SYNTHESIS OF HEPATITIS C VIRUS RNA TARGETED THERAPEUTICS AND STUDIES TOWARDS THE EPIDITHIODIKETOPIPERAZINE ALKYLATION IN CHAETOMIN

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Hepatitis C virus (HCV) infection has become a leading killer worldwide, affecting more than 170 million people and leading to cirrhosis of the liver, end-stage liver disease, and hepatocellular carcinoma. Current therapies for HCV are associated with significant side effects, as well as increased resistance. Therefore, there is a clear need for a less toxic and more effective therapy. RNA is becoming an important therapeutic target, despite the challenge associated with developing molecules targeting RNA. The HCV internal ribosome entry site (IRES) located at the 5' untranslated region of the viral RNA has become a most attractive therapeutic target. Domain II of the HCV IRES has a 90^{o} bent helical structure, which is crucial for the function of the IRES. Benzimidazole based HCV replication inhibitors that exert their activity by altering the structure of the domain IIa of the HCV IRES have been reported.

The Rainier group, in collaboration with the Davis, Cheatham and Hagedorn groups of the University of Utah, plan to reach the goal of developing efficient HCV therapeutics. The plan centers around utilizing the previously reported synthesis of quaternary substituted indolines and thiopyranylindolines by Rh(II) mediated diazo decomposition to examine the scope of potential HCV inhibitors. A serotonin derivative was coupled with a vinyl diazo ester in the presence of $Rh_2(OAc)_4$ to provide a quaternary substituted indoline, which after cyclization with TFA provided the pyrroloindoline based potential HCV inhibitors.

Chaetomin was isolated from *Chaetomium* sp. and was found to possess antibiotic effects, immunomodulatory activity, and anti cancer activity. Thus, the fascinating architecture, as well as its striking biological activity, have made chaetomin an attractive synthetic target. The indole-diketopiperazine bridge in chaetomin is an important structural feature of its northern hemisphere. The bridgehead carbanion in the epidithiodiketopiperazine moiety was quenched with a skatole derivative to produce an indole-diketopiperazine coupled product.

Outlined here are the studies directed toward the synthesis of pyrroloindoline based potential HCV inhibitors, and the synthesis of the indole-diketopiperazine bridge in chaetomin.

To the most wonderful people in my life,

my parents, sister and Aravinda,

for their unconditional love and support

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LIST OF ABBREVIATIONS

ABSA	4-acetamidobenzenesulfonyl azide
Ac	acetyl
Boc	*t*-Butoxycarbonyl
Bu	butyl
Bz_2O_2	benzoyl peroxide
^oC	celsius degrees
calc'd	calculated
CSA	camphorsulfonic acid
d	doublet (spectral)
d. r.	diastereomeric ratio
DCM	dichloromethane
dd	doublet of doublet (spectral)
ddd	doublet of doublet of doublet (spectral)
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIPEA	diisopropylethylamine
DKP	2,5-diketopiperazines
DMA	N, N-dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
dt	doublet of triplet (spectral)
DTAD	di-t-butyl azodicarboxylate

DTNB	Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid))
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
ETP	epipolythiodioxopiperazines
FT-IR	fourier transform infrared
g	gram(s)
h	hour(s)
HCV	hepatitis C virus
HIF-1α	hypoxia inducible factor-1 α
HIV	human immunodeficiency virus
HPLC	high pressure liquid chromatography
^{i}Bu	iso-butyl
IRES	internal ribosome entry site
LAH	lithium aluminum hydride
LHMDS	lithium hexamethyldisilazide
LRMS (ESI)	low resolution mass spectroscopy (electrospray ionization)
m	multiplet (spectral)
m-CPBA	meta-chloroperbenzoic acid
Me	methyl
MeOH	methanol
min	minute(s)
ml	mililitre
mol	mole(s)
MsCl	methanesulfonyl chloride
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance

Ns	p-nitrobenzenesulfonyl
ppm	parts per million
PTLC	preparative thin layer chromatography
q	quartet (spectral)
quant.	quantitative yield
R_f	retention factor (chromatography)
RNA	ribonucleic acid
RT	room temperature
s	singlet (spectral)
t	triplet (spectral)
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBDMS (TBS)	t-butyldimethylsilyl
TBSP	tetrakis{1-[(4-tert-butylphenyl)sulfonyl]-(2S-pyrrolidinecarboxylate})
^{t}Bu	tert-butyl
td	triplet of doublet (spectral)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSI	trimethylsilyl iodide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TPP	meso-tetraphenylporphyrin
Ts	p-toluene sulfonyl
UTR	untranslated region
UV	ultraviolet spectroscopy

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CHAPTER 1

SYNTHESIS OF HEPATITIS C VIRUS RNA TARGETED THERAPEUTICS

Introduction

Hepatitis C virus (HCV) infection has become a highly demanding global healthcare burden, with the likelihood of increase in the near future. More than 170 million people (approximately 3% of the world's population) are infected with HCV. In addition, approximately 3–4 million new cases of HCV infection are discovered each year.[1] Unfortunately, most infections become chronic; a condition that is incurable in many patients, leading to cirrhosis, end-stage liver disease, and hepatocellular carcinoma. In developed countries, HCV infection is responsible for 50–76% of all cases of liver cancer and for two-thirds of all liver transplants. Since the acute infection is usually asymptomatic, early diagnosis has become difficult, which has increased the tendency towards chronicity. Consequently, HCV infection has become a leading killer worldwide and the most common cause of liver failure in United States.[2]

A common feature of HCV, hepatitis B, and human immunodeficiency (HIV) viruses is that they are primarily transmitted percutaneously. Before the screening of blood for HCV, HCV is mostly spread by blood transfusions, blood related products, haemodialysis, and organ transplantations. Nowadays HCV is mainly spread by

injecting drug users, and to a much lesser extent, via sexual and perinatal transmission.[1] HCV has become a significant problem in correctional facilities, where 20–40% of inmates are infected.[2] In fact, a significant amount of patients infected with HIV are co-infected with HCV, which increases the risk of cirrhosis.

The hepatitis C virus is a noncytopathic, hepatotropic member of the Flaviviridae family, whose primary target organ and cell are the liver and the hepatocyte respectively. HCV triggers a strong innate intracellular immune response in host cells as it spreads, but resists the function of the antiviral target genes that it induces. This immune response is similar in animals that overcome the infection as well as those that become persistently infected, implying that any influence on the outcome is indirect or obscure. However, viral strategies to circumvent the innate intracellular immune response, suggests its importance in controlling HCV infection. In addition, the outcome of HCV infection is mainly determined by the magnitude, diversity, and quality of the adaptive immune response.[2]

The outcome of HCV infection is also determined by several viral factors, such as HCV genotypes, replication and mutation rates. Six distinct genotypes of HCV have been identified that show marked differences in geographic distribution, disease progression, and response to therapy. Genotype I is the most prevalent viral strain in Europe and North America. The mutation rate, as well as the replication rate of HCV is high, which results in an explosive expansion of the numerous viral populations after inoculation.[2]

The HCV genome was first cloned by Houghton and colleagues in 1989. It is a 9.6-kilobase uncapped linear single-stranded RNA molecule with positive polarity. The 5'and 3' end of HCV RNA contain untranslated regions (UTRs), which include control

elements required for translation and replication. HCV RNA genome is translated into a polyprotein of approximately 3,000 amino acids, comprising of four structural (C, E1, E2 and p7) and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Figure 1.1).[1,2]

In developing drugs targeting HCV, researchers have focused on inhibiting different steps in the life cycle of the virus, which is entirely cytoplasmic. These small-molecule, orally bio-available drugs function as inhibitors of the HCV enzymes, viral RNA, as well as in modulating the host immune response. Among all HCV enzymes, the NS3-4A serine protease and the NS5B RNA polymerase have emerged as the most popular therapeutic targets.[3]

HCV infects only humans and chimpanzees. Thus, research on HCV was hampered by the lack of small animal models, as well as lack of a robust cell-culture system until 2005. This new robust HCV infection system is based on a unique HCV genome (JFH1) derived from the blood of a Japanese patient with fulminant hepatitis, and shows extraordinary replication in vitro.[2,4]

The standard treatment for chronic HCV infection involves a combination of pegylated IFN (interferon) and ribavirin, which is also given for patients co-infected with HCV and HIV. It has been observed that there is a delayed response in the co-infected patients, which could be due to the higher viral load and lower immunity in co-infected patients. It is implied that the activity of this therapy is due to its anti viral effect as well as immunostimulatory effect. However, the therapy is associated with significant side effects in some patients, as well as increased resistance.[1,4] Therefore, there is a clear need for a less toxic and more effective therapy.

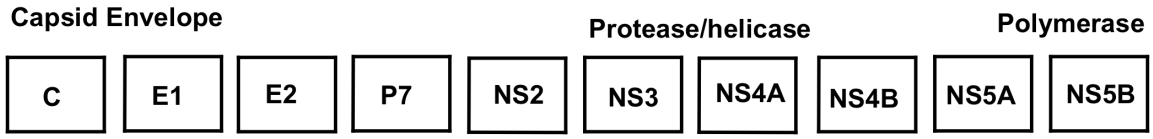


Figure 1.1. Genomic organization of proteins of HCV

The major roadblock in HCV treatment is the rapid emergence of drug-resistant viruses under the selective pressures exerted by antiviral drugs. Usually in each new HCV genome, there is an average of one nucleotide change per replication cycle. Therefore, HCV exists as a genetically heterogeneous viral population, known as a "quasispecies," even in untreated individuals. Thus, a successful drug candidate should have the ability to be effective on all HCV genotypes, as well as suppress mutations. As in the case of HIV, a combination of multiple drugs, directed at both viral and host targets, would be more promising.[3] Due to the tremendous effort of researchers, an effective therapy for HCV may be within reach.

Background

RNA is becoming an important therapeutic target due to our increasing knowledge of the vast role played by RNA in biochemical processes and disease progression.[5] For instance, Erythromycin, an antibiotic in clinical practice, binds ribosomal RNA; also, RNA is the genetic material of the HIV and hepatitis C virus. Aminoglycoside antibiotics are the initial RNA targeted therapeutic, but these lack selectivity. Therefore, it's worth searching for small molecules that bind to RNA targets more efficiently and selectively. However, developing molecules targeting RNA has become extremely challenging due to the lack of understanding of RNA recognition

principles.[5] In addition, due to the heterogenous nature of RNA, it's difficult to crystallize and characterize.[6] Furthermore, our understanding of drug RNA interactions is made difficult by the remodeling of RNA structure upon drug binding, due to the inherent flexibility of nucleic acids.

The HCV internal ribosome entry site (IRES) located at the 5' UTR of the viral RNA (Figure 1.2) has become a most attractive target, due to the structural and functional data available and also because of the conservation of IRES among HCV genotypes.[3] These IRES structures are also found in viral pathogens such as polio, foot-and-mouth disease, and swine fever virus.[5] IRES mediates the initiation of viral-RNA translation in a cap-independent manner, and is also important for HCV RNA replication.[7] Domain II of the HCV IRES has a 90^{o} bent helical structure that is conserved in HCV and related viruses such as swine fever virus, and appears to be essential for the function of the IRES.[8]

A series of small-molecule HCV replication inhibitors based on a benzimidazole core has been reported.[7] These molecules bind to the domain IIa of the HCV IRES, with low micromolar affinities. Mass spectrometry-based high throughput screening led to the identification of the benzimidazole hit **1.1** (Figure 1.3) with modest affinity and selectivity against the domain IIA 29-mer. In their attempts to optimize the structure, Seth et al.[7] found that the dimethylamino head group on the tether at N1 to be crucial for RNA binding. When the dimethylamino group was replaced by other cationic head groups such as pyrrolidino, diethylamino and morpholino slight to substantial losses of binding affinity were observed.[7]

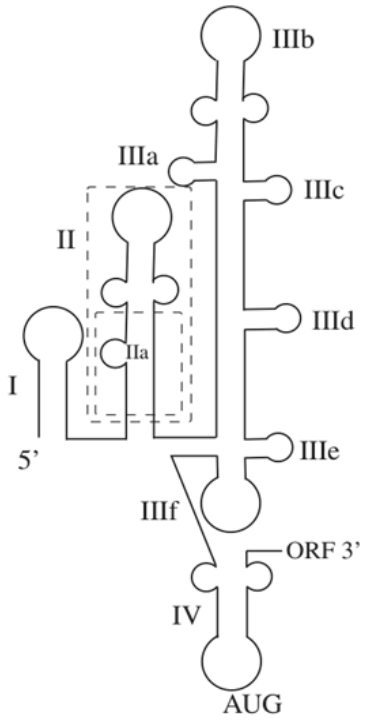


Figure 1.2. Structure of HCV IRES RNA[8]

In their attempts to modify the benzimidazole core, Seth et al.[7] realized that a methoxy group at C6 in **1.2** resulted in a slight improvement of the binding affinity. Furthermore, introduction of a 3-(dimethylamino)propyloxy side chain at C6 in **1.3** resulted in roughly 10-fold increase in binding affinity and a 5-fold increase in selectivity. They found out that the increase in binding affinity is due to the 2-amino group and the cationic side chains at N1 and C6, involving specific interactions with the RNA. Constraining the C6 side chain and N1 side chain to the benzimidazole core, shown in **1.4**, **1.5** and **1.6**, resulted in a spectacular increase in binding affinity. In addition, these molecules reduced HCV RNA levels at low micromolar concentrations in an HCV replicon assay.[7]

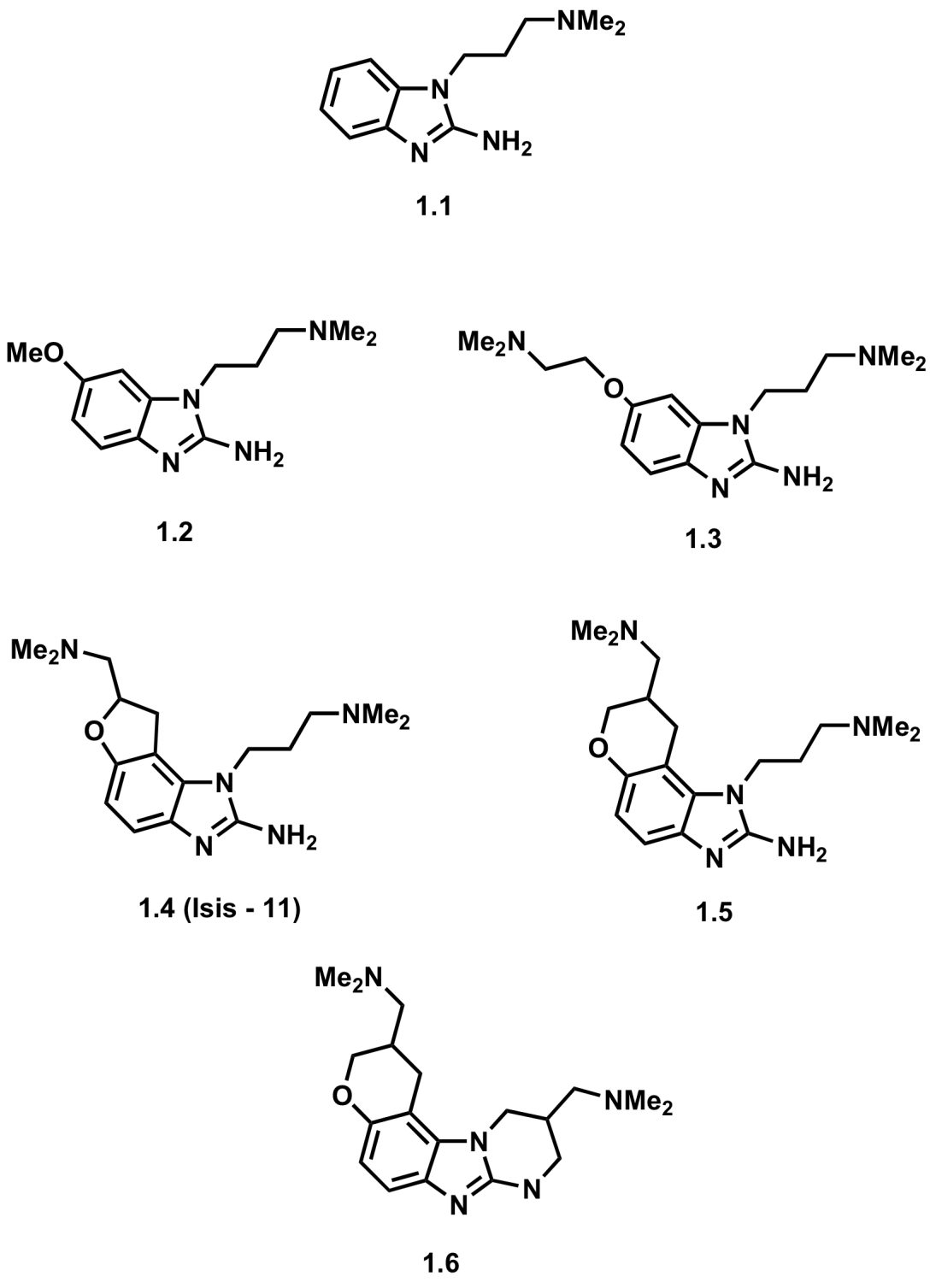


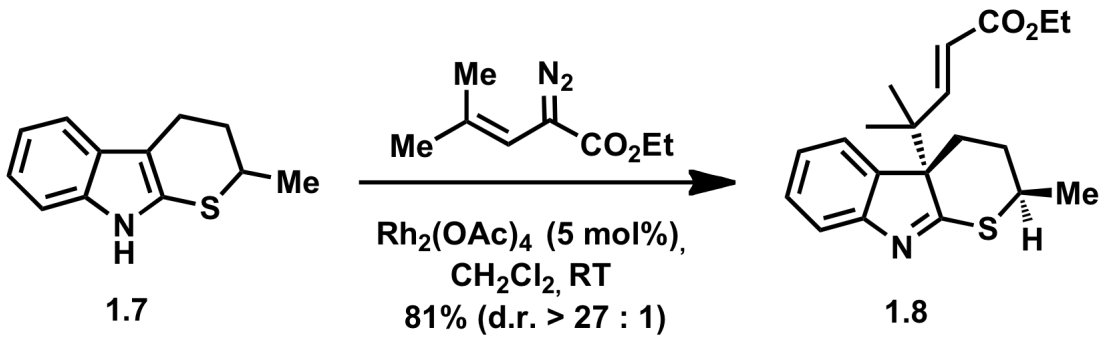
Figure 1.3. Benzimidazole based HCV inhibitors

Studies[8] on the mechanism of action of the benzimidazoles revealed that HCV replication inhibition is due to the changes in IRES structure on inhibitor binding. Isis-11 (**1.4**) binds specifically to the domain IIa bulge, displacing key nucleotide residues within the bulge region and affecting the stacking interactions, which ultimately results in a major structural reorganization. As it has been observed in solution and crystal form, inhibitor bound RNA structure has a straight form, in contrast to the bent form of free RNA. This structural change is further evident from large chemical shift changes in the NMR spectra of the inhibitor and conserved bulge region of RNA in both free and bound states. In addition, fluorescence intensity changes were measured in the modified bulge region, in which adenosine residues were replaced with 2-aminopurine. Inhibitor binding results in a large increase in fluorescence in the 5' end, due to the interruption of stacking interactions, whereas quenching of fluorescence in the 3' end suggests new stacking interactions. The investigations on the crucial functional groups of benzimidazole based HCV inhibitors revealed (from crystal structures) that the two dimethylamino groups replace two discrete metal ions in the divalent metal sites of RNA. The benzimidazole ring acts as a rigid linker for the two dimethylamino chains and involves RNA binding via π-stacking. Interestingly, the inhibitor binds with low micromolar affinity despite lacking any specific hydrogen bonding interactions, opening up the way to further optimize these molecules to create a new class of highly potent and specific HCV therapeutics.[8]

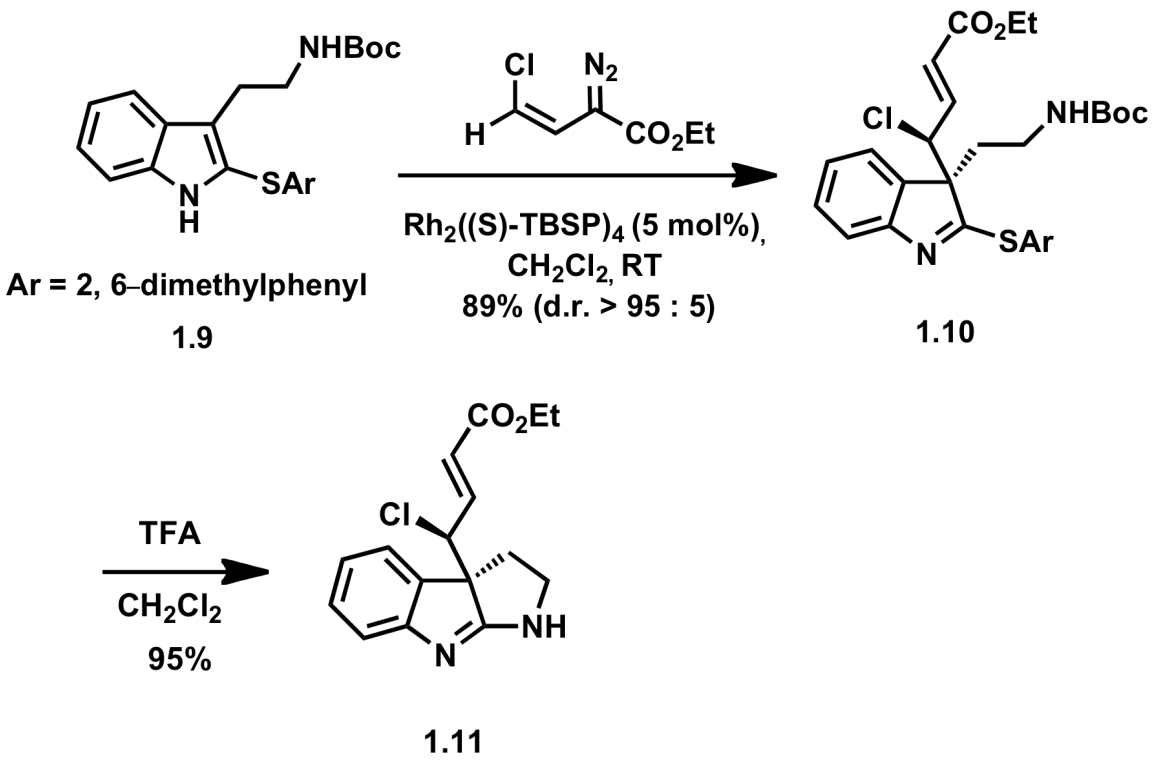
The Rainier group, in collaboration with Davis, Cheatham and Hagedorn groups[9] of University of Utah, plans to reach the goal of developing novel, efficient, small molecule HCV therapeutics. The Rainier group plans on increasing the scope of potential

HCV inhibitors by synthesizing molecules centered around indoles, quaternary substituted indolines, and quaternary substituted thioindolines.

The Rainier group has previously reported the synthesis of quaternary substituted indolines and thiopyranylindolines by Rh(II) mediated diazo decomposition. For instance, thiopyran **1.7** was coupled with vinyl diazoester in the presence of $Rh_2(OAC)_4$ to give the corresponding thiopyranylindoline **1.8** in high yield and high diastereoselectivity (Scheme 1.1).[10] Similarly, 2-thioindole **1.9** was reacted with vinyl diazoester in the presence of catalytic amount of Rh(II) to give indoline **1.10** from a [3,3]-sigmatropic rearrangement (Scheme 1.2).[11] Subsequent reaction with TFA gave the desired pyrroloindoline **1.11**. These reactions are in general high yielding, highly stereoselective and tolerate different substitution patterns, giving rise to a rich array of substrates. Thus we were hopeful that we could create a library of promising HCV inhibitors. The first chapter of this thesis is focused on the studies directed towards the synthesis of potential HCV inhibitors, based on quaternary substituted indolines.



Scheme 1.1. Thiopyranylindoline synthesis



Scheme 1.2. Pyrroloindoline synthesis

Reterosynthesis

Our initial intention was to synthesize a series of potential inhibitors centered on pyrroloindoline **1.12** (Figure 1.4). Our targeted indoline compounds were docked into the RNA binding site (Figure 1.5) by Dr. Tom Cheatham,[9] and proved to be promising HCV inhibitors. From the molecular docking studies, our targeted indoline compound **1.12.a** has a score value of -13.2 kcal/mol compared to -10.4 kcal/mol of the benzimidazole compound **1.13** (Figure 1.6). These values are comparable with the known benzimidazole based inhibitors[7], which compelled us to investigate the synthesis of a library of inhibitors based on pyrroloindoline **1.12**.

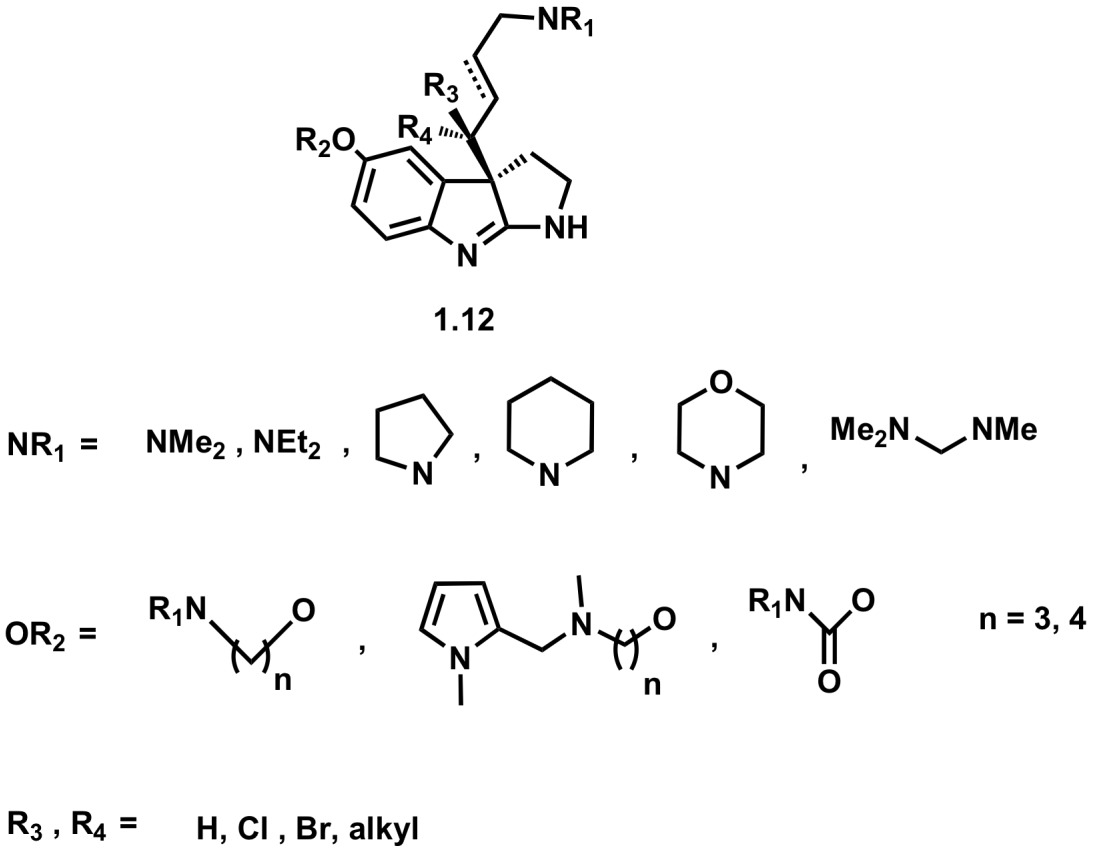


Figure 1.4. Targeted pyrroloindoline based HCV inhibitors

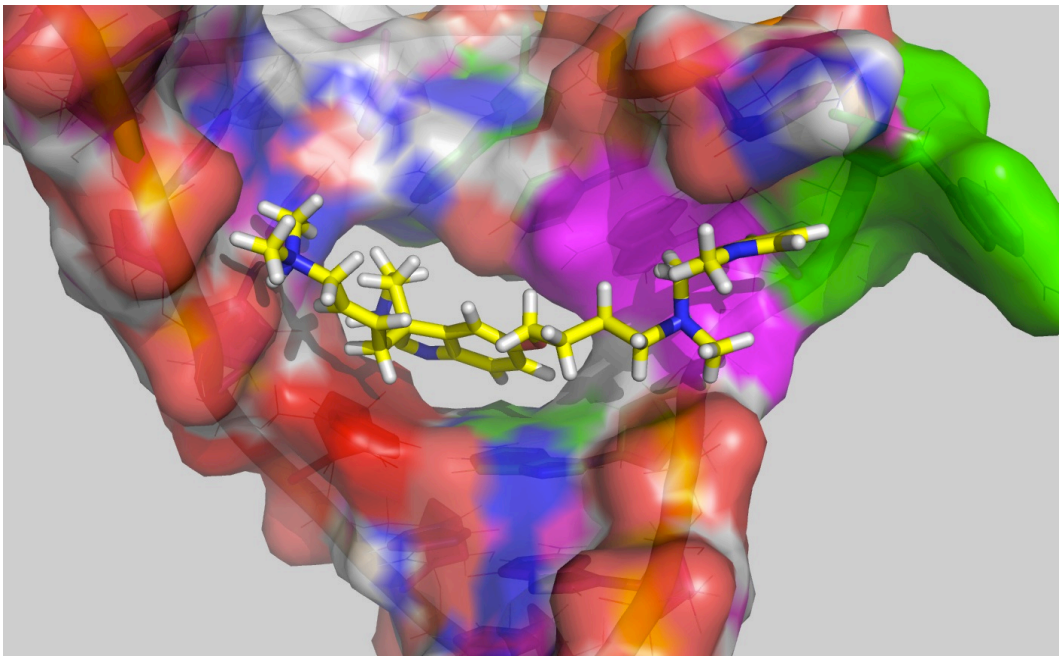


Figure 1.5. Targeted indoline compounds docked into the RNA binding site

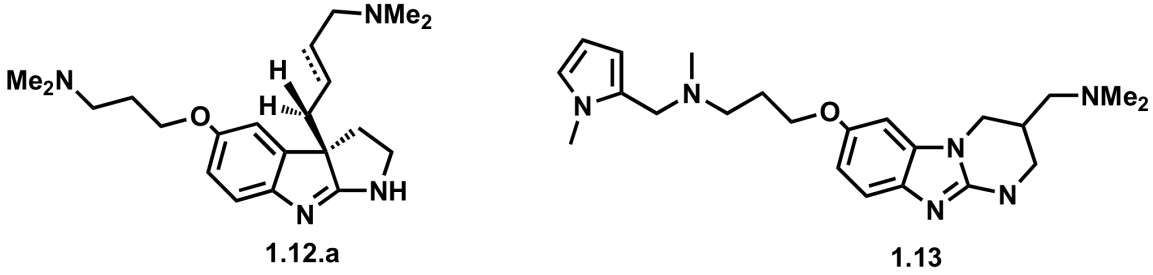


Figure 1.6. Molecular docking score values of potential HCV inhibitors

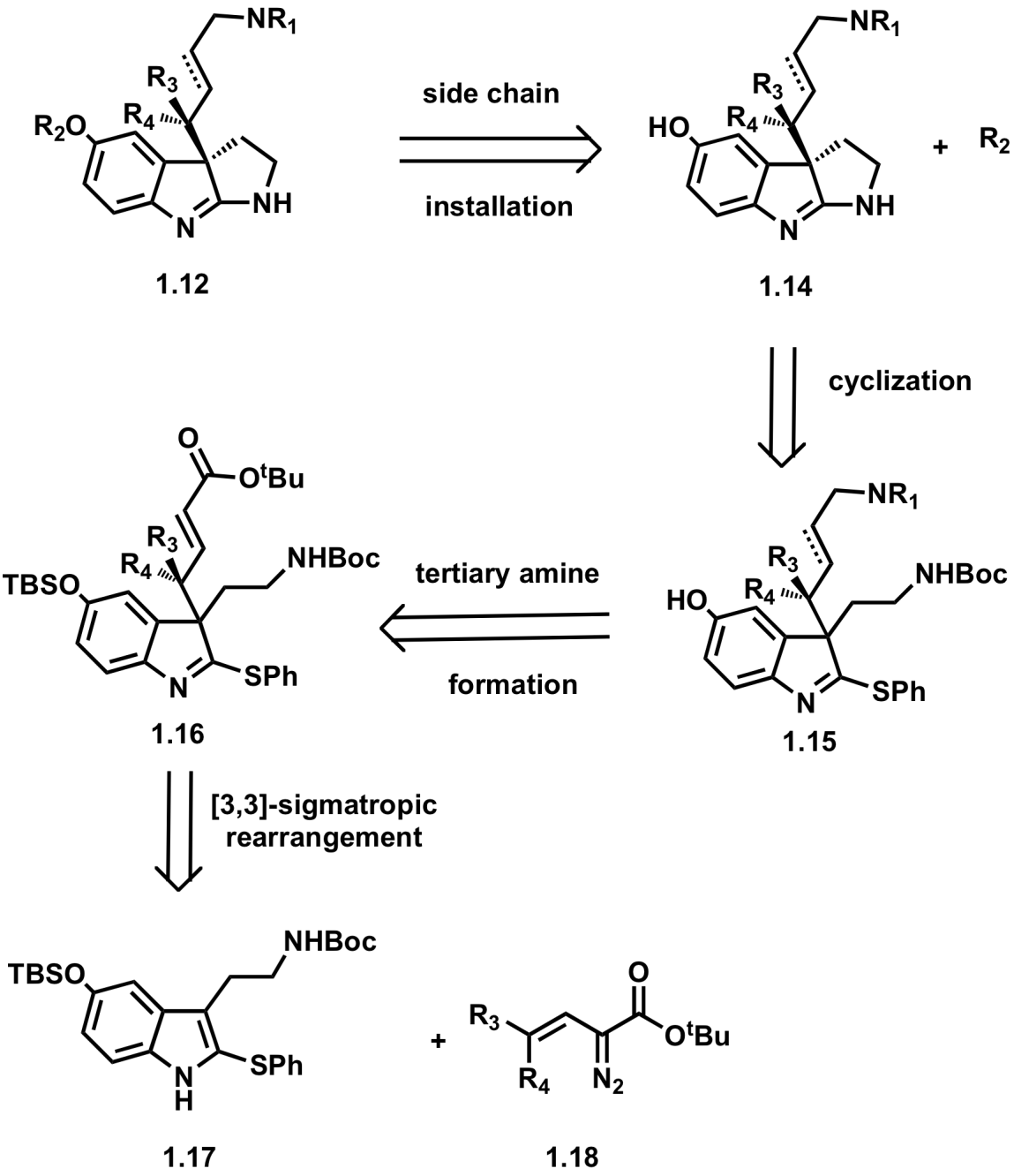
It was envisioned that **1.12** would arise from the final side chain installation by phenolic etherfication or carbamoylation between **1.14** and the respective side chain $\mathbf{R_2}$ (Scheme 1.3). Deprotection of the Boc group of thioindoline **1.15** and subsequent cyclization would provide pyrroloindoline **1.14**. The tertiary amine group of **1.15** would arise from functionalization of vinylic ester in **1.16**. Rh(II) mediated [3,3]-sigmatropic rearrangement between 2-thioindole **1.17** and vinyl diazoester **1.18** would give thioindoline **1.16**.

Results and Discussion

Studies towards synthesis of pyrroloindoline core

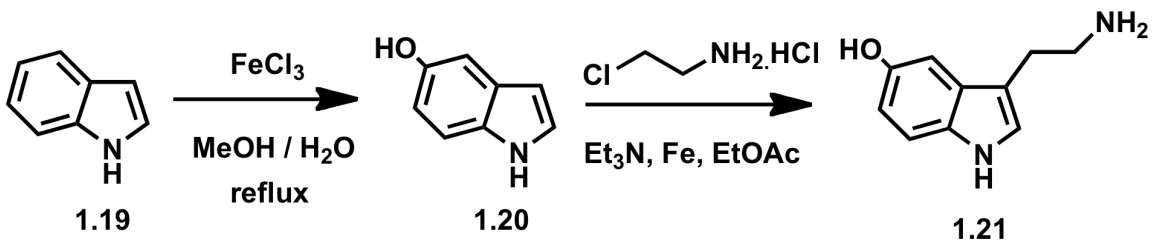
Our initial goal was to synthesize serotonin. Several attempts to synthesize serotonin have been reported in the literature, including biotransformations, long sequence synthesis, and a concise synthesis by Yang and Cao (Scheme 1.4).[12] When we were attempting to follow their procedure, we were unable to reproduce their initial step of oxidation of indole **1.19** to 5-hydroxyindole **1.20**. Then the second step of alkylation of **1.20** with chloroethylamine hydrochloride, to give serotonin **1.21**, was attempted with commercially available 5-hydroxyindole, which was also unsuccessful. Protection of 5-hydroxy group of **1.20** with TBSCl and subsequent alkylation attempts only led to the recovery of the starting material.

Synthesis of tryptamine and tryptamine derivatives by reduction of 3-indoleglyoxylamide has been reported[13] previously (Scheme 1.5). The approach by Woodward,[13a] Vanderwerff,[13b] and Stoltz[13c] involved synthesis of 3-indoleglyoxalyl chloride by reaction of indole **1.22** with oxalyl chloride.

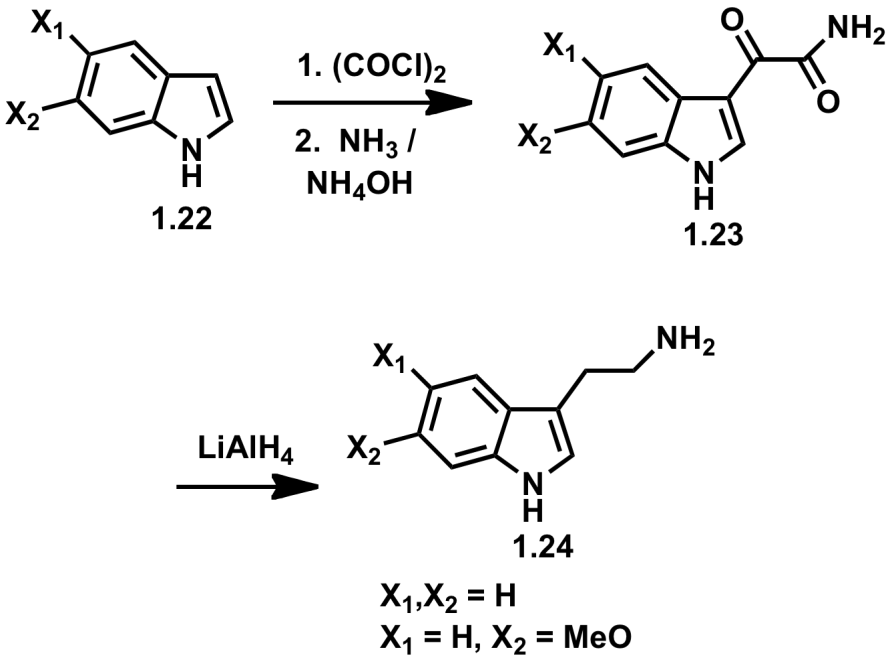


R_1, R_2, R_3, R_4 = as in Figure 1.4

Scheme 1.3. Reterosynthetic analysis



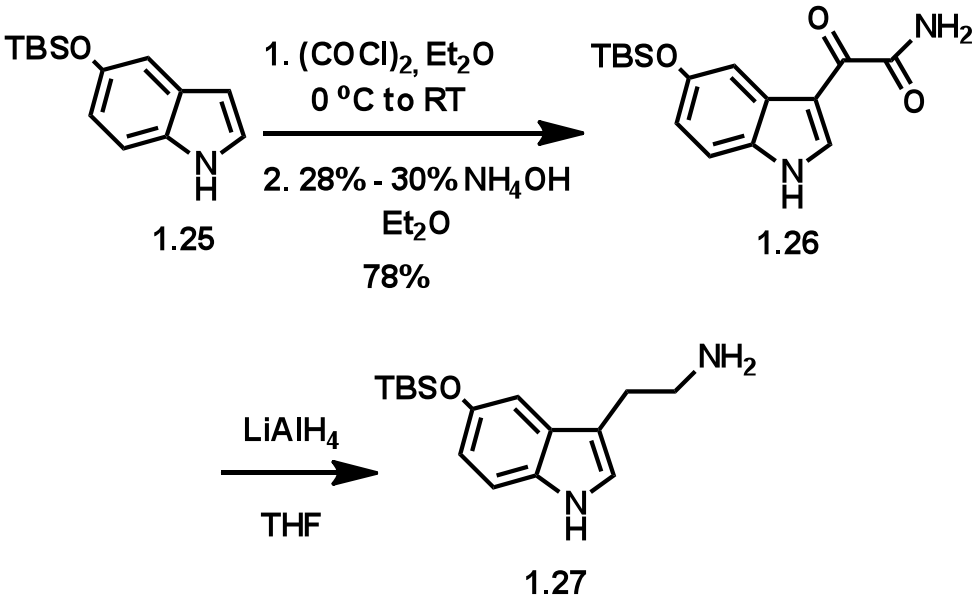
Scheme 1.4. Synthesis of serotonin by Yang and Cao



Scheme 1.5. Synthesis of tryptamine derivatives

Subsequently, the glyoxalyl chloride was converted into the corresponding primary amide to provide 3-indoleglyoxylamide **1.23**. Reduction with $LiAlH_4$ gave tryptamine **1.24** (Scheme 1.5).

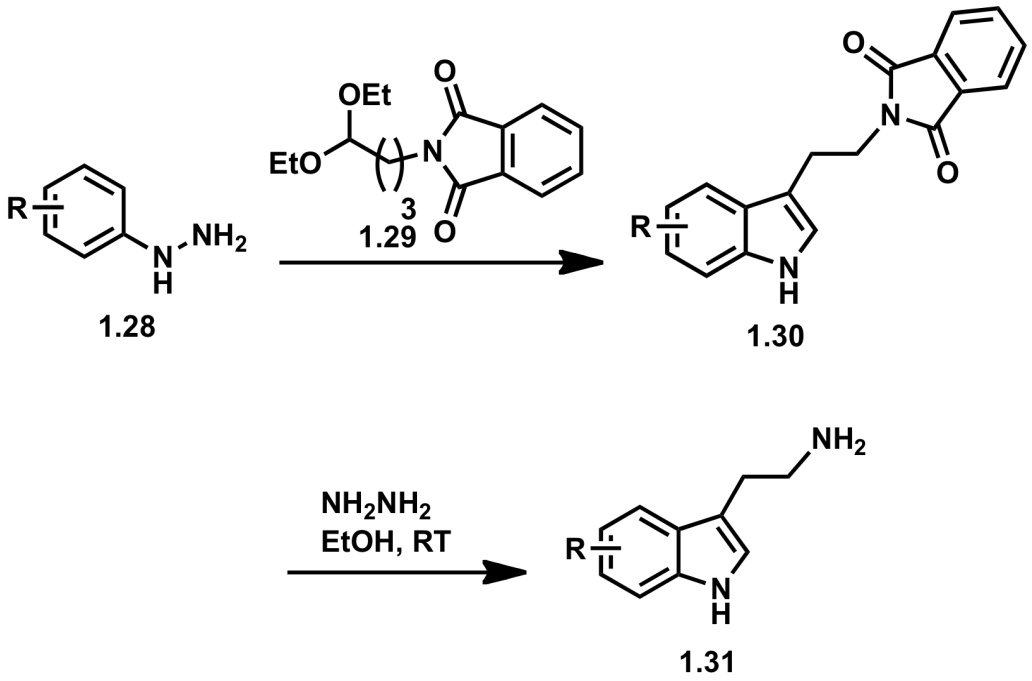
Our attempt to synthesize serotonin using the above method involved conversion of 5-hydroxyindole **1.25** into indoleglyoxylamide **1.26** in 78% yield (Scheme 1.6). Subsequently, reduction of **1.26** with $LiAlH_4$ gave a mixture of the starting material **1.26** and serotonin **1.27** from the crude NMR. Unfortunately, it was difficult to reproduce the reduction step. Thus we started our synthesis with commercially available serotonin due to the time restraints.



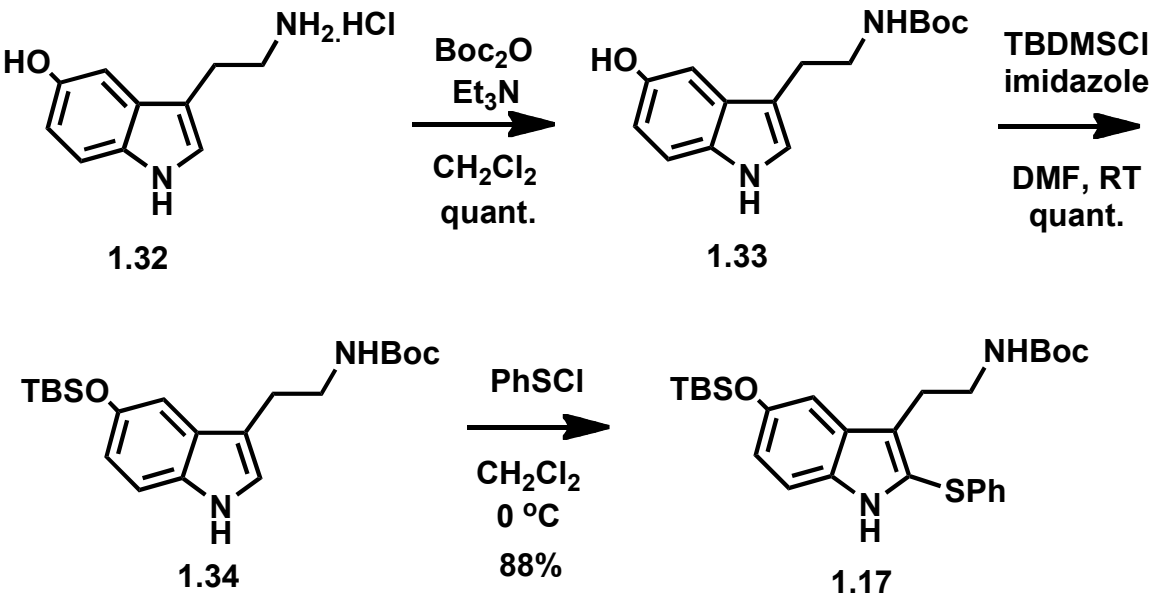
Scheme 1.6. Synthesis of serotonin via indoleglyoxylamide

During the course of our synthesis, Barsanti et al.[14] reported a concise approach for the synthesis of tryptamine derivatives using Fischer indole synthesis. They utilized phenyl hydrazine derivatives **1.28** with bis-ethoxyacetal **1.29**, to give phthalimide protected amine **1.30**, which on deprotection, using hydrazine, afforded the tryptamine derivatives **1.31** (Scheme 1.7).

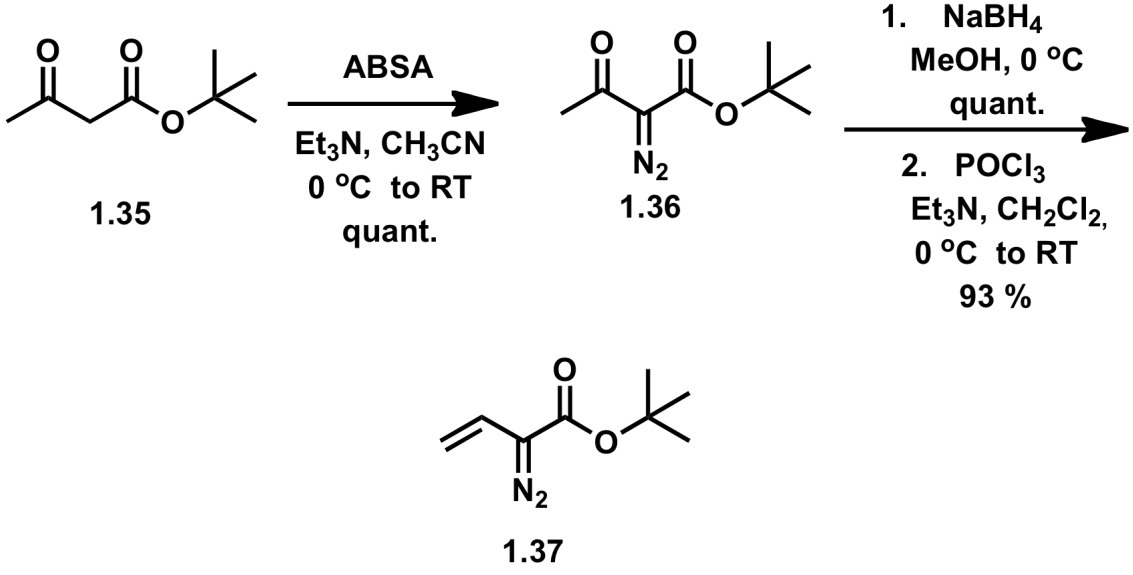
Initially, Boc protection of serotonin hydrochloride **1.32** afforded **1.33**, which after TBS protection provided **1.34** (Scheme 1.8). Then, **1.34** was reacted with PhSCl to give 2-thioindole, **1.17**. Vinyl diazo compound was prepared from t-butylacetoacetate **1.35** in three steps (Scheme 1.9).[15] Reaction of **1.35** with diazo transfer reagent ABSA gave **1.36**. $NaBH_4$ reduction of **1.36** gave the corresponding secondary alcohol, which on dehydration with $POCl_3$ provided the vinyl diazo compound **1.37**.



Scheme 1.7. Synthesis of tryptamine derivatives by Barsanti et al.



Scheme 1.8. Synthesis of serotonin 2-thioindole derivative

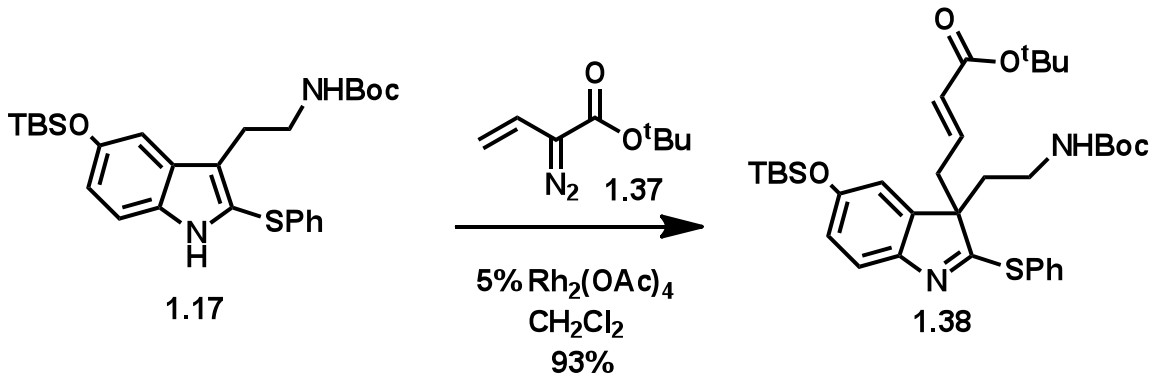


Scheme 1.9. Synthesis of vinyl diazoester

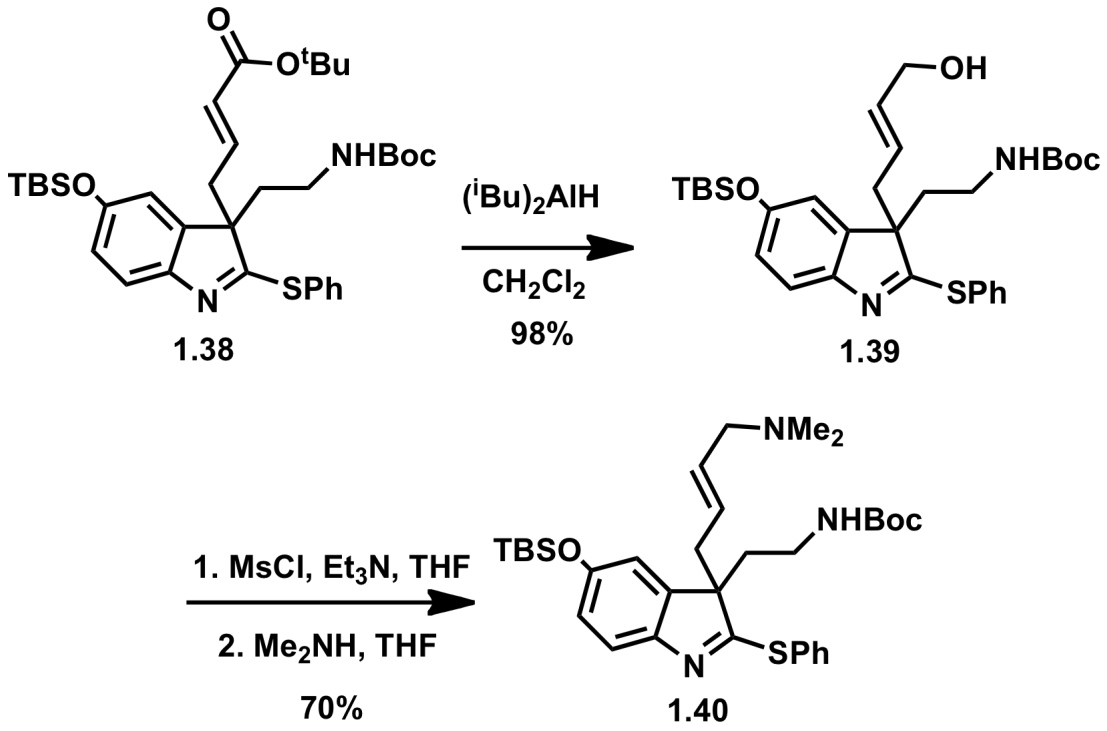
2-Thioindole **1.17** was reacted with vinyl diazo compound **1.37** in the presence of catalytic $Rh_2(OAc)_4$, to give indoline **1.38** from a [3,3]-sigmatropic rearrangement (Scheme 1.10). Although **1.37** is known to be unstable, we were thrilled at the high yields observed when freshly prepared **1.37** was slowly added to the reaction. In our attempts to scale up the reaction (up to 3 g scale of **1.34**), we were pleased by the fact that yields were unaffected.

Vinylic ester compound **1.38** was reduced by $(^{i}Bu)_2AlH$, to give the the allylic alcohol compound **1.39**. In order to make the allylic tertiary amine compound **1.40**, **1.39** was reacted with MsCl to form the corresponding allylic mesylate, which was subsequently reacted with dimethylamine to give **1.40** (Scheme 1.11).[16]

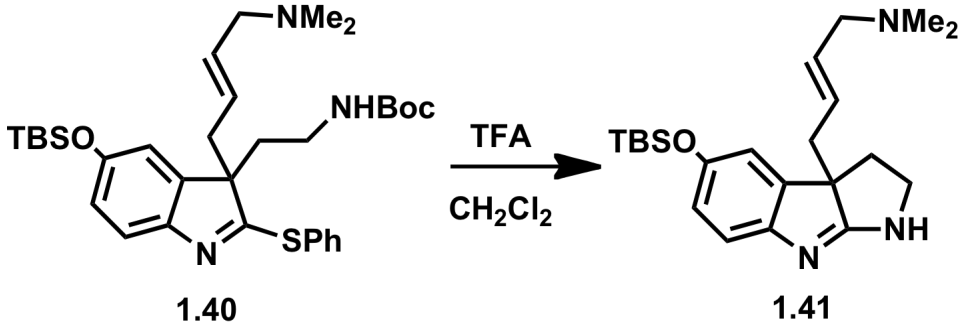
Pyrroloindoline **1.41** could be obtained by Boc group removal and the subsequent cyclization of thioindoline **1.40** (Scheme 1.12). In fact, similar cyclizations have been reported previously by the Rainier group (Scheme 1.2).[11] For example, the NaH mediated cyclization of thioindoline **1.42** provided pyrroloindoline **1.43** and **1.45** (Scheme 1.13).[17]



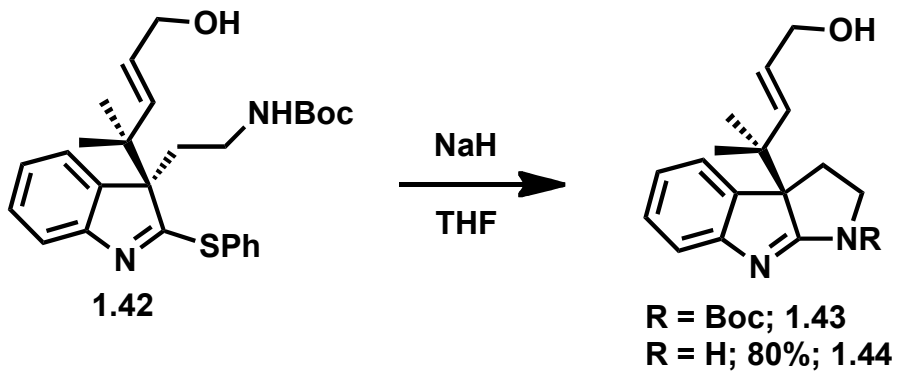
Scheme 1.10. Synthesis of thioindoline from diazo decomposition



Scheme 1.11. Synthesis of allylic dimethylamine compound

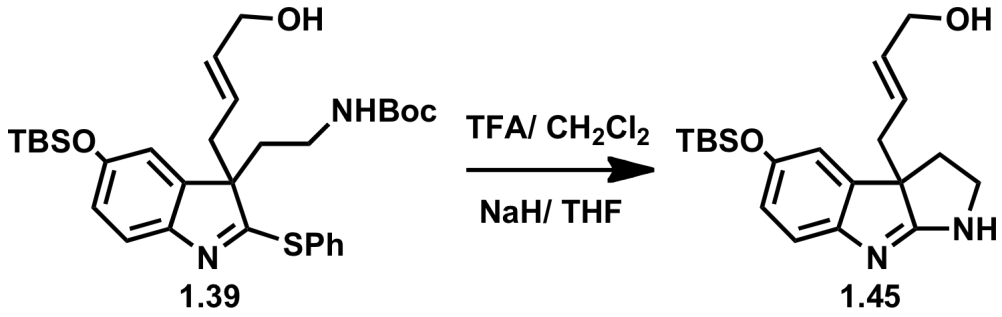


Scheme 1.12. TFA mediated cyclization to form pyrroloindoline



Scheme 1.13. NaH mediated cyclization to give pyrroloindoline

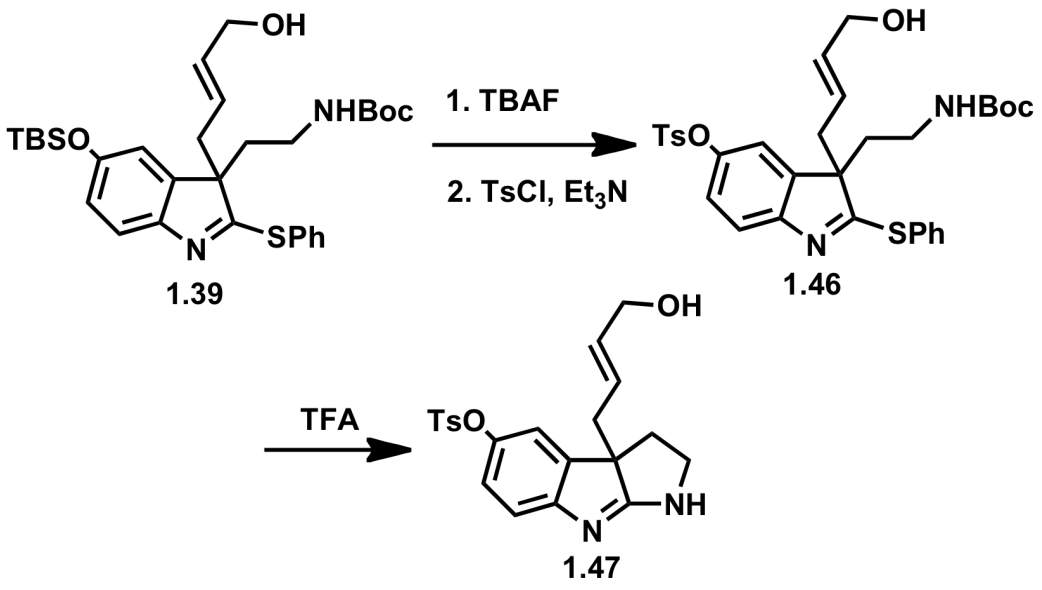
To our surprise, when we attempt to cyclize **1.40**, we only isolated an unidentified compound. After comparing **1.40** with the previous substrates **1.10** and **1.42**, we came to the conclusion that the tertiary amine was the problem. Therefore, cyclization was attempted with the allylic alcohol substrate **1.39** to give **1.45**, under both TFA and NaH conditions (Scheme 1.14). To our disappointment, in both attempts, instead of **1.45**, we were still able to isolate the same unidentified compound. Attempts to cyclize the vinyl ester compound **1.38** were also unsuccessful.



Scheme 1.14. Attempted cyclizations to obtain pyrroloindoline

When comparing **1.39** with **1.42**, other than the two methyl groups, the major difference is the 5-OH substituent in **1.39**. Thus, with the thought that the activation of the ring by the hydroxyl substituent leads to the unexpected observations, we decided to protect the phenol with an electron withdrawing protecting group. Removal of the TBS group from **1.39**, protecting with a tosyl group, gave compound **1.46**. Unfortunately, attempts to cyclize **1.46** with TFA to give **1.47** were not successful either (Scheme 1.15).

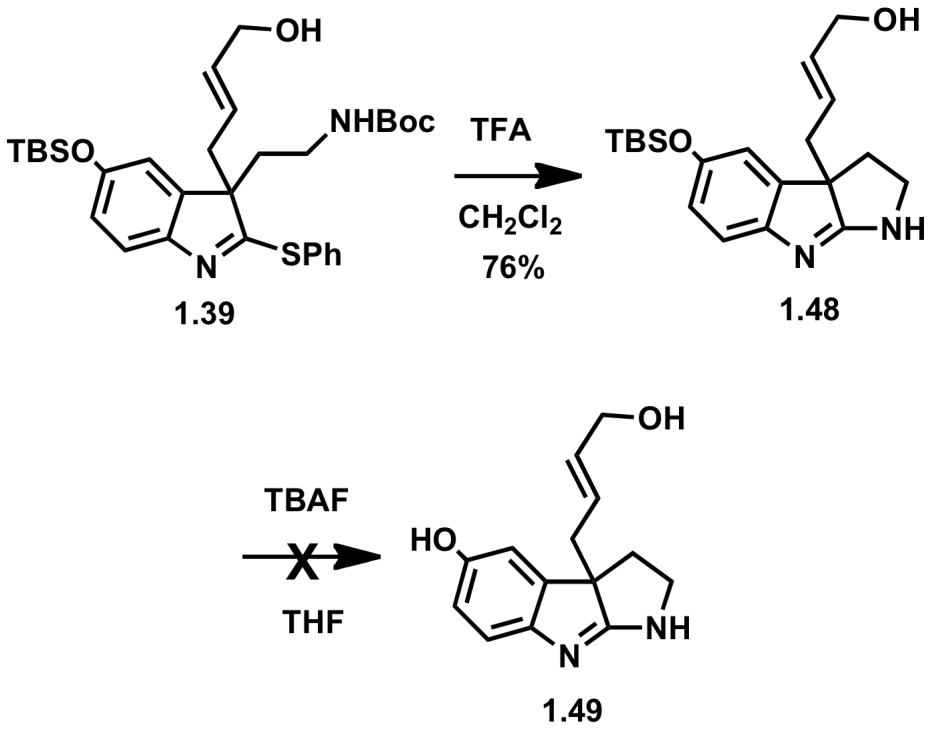
More than a dozen times to cyclize under different conditions were attempted using **1.39**. For example, instead of TFA to remove the Boc group, Lewis acids like TMSI, TMSOTf and $SnBr_2$ and also heat were used. Although we were not able to successfully isolate the desired compound, it was possible to observe the product in the crude ^1H NMR when a nonaqueous work up was carried out after the reaction, or when Amberlyst-21 was used to quench the reaction.[18]



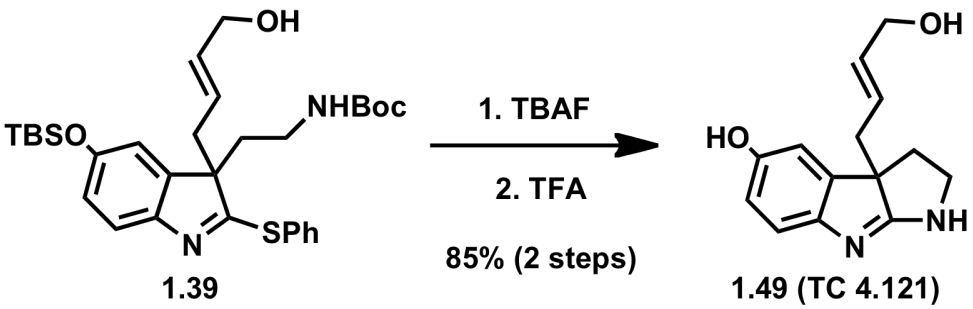
Scheme 1.15. Attempted cyclization on tosyl protected thioindoline

Finally, the amount of aqueous $NaHCO_3$ used in the neutralization process during the work up proved to be critical. When a large excess was used, no product was isolated. When small quantities were used, **1.48** was isolated in 76% yield (Scheme 1.16). Attempts to deprotect the TBS group in **1.48** to give **1.49** led to the same unidentified compound that was observed previously.

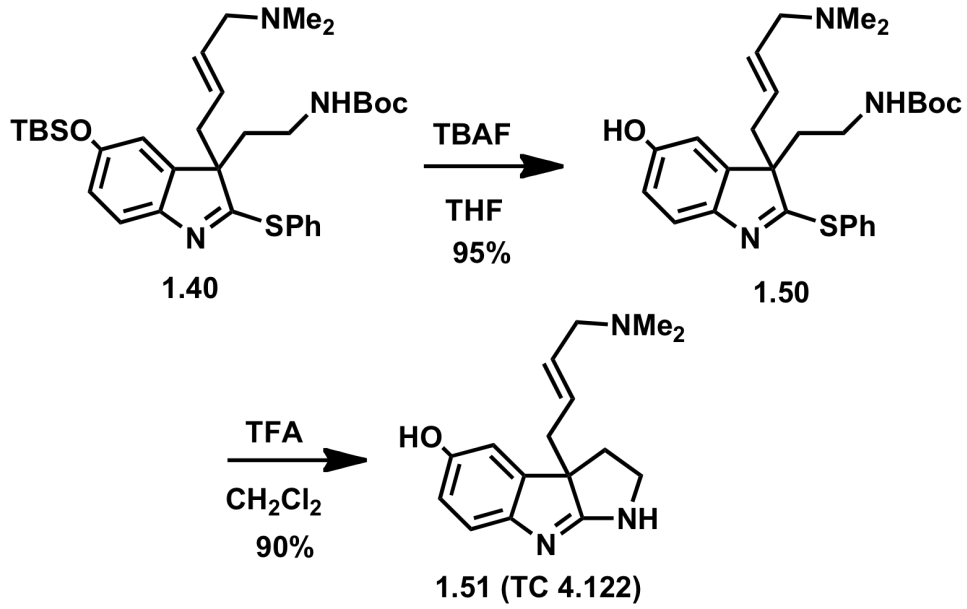
Interestingly, deprotection of the TBS group of **1.39** and subsequent cyclization resulted **1.49** (**TC 4.121**) in 85% yield (Scheme 1.17). Inspired by this outcome, we subjected **1.40** to TBAF to afford **1.50** in a 95% yield and cyclization with TFA provided **1.51** (**TC 4.122**) (Scheme 1.18).



Scheme 1.16. Synthesis of pyrroloindoline via TFA mediated cyclization



Scheme 1.17. Synthesis of pyrroloindoline **TC 4.121**



Scheme 1.18. Synthesis of pyrroloindoline **TC 4.122**

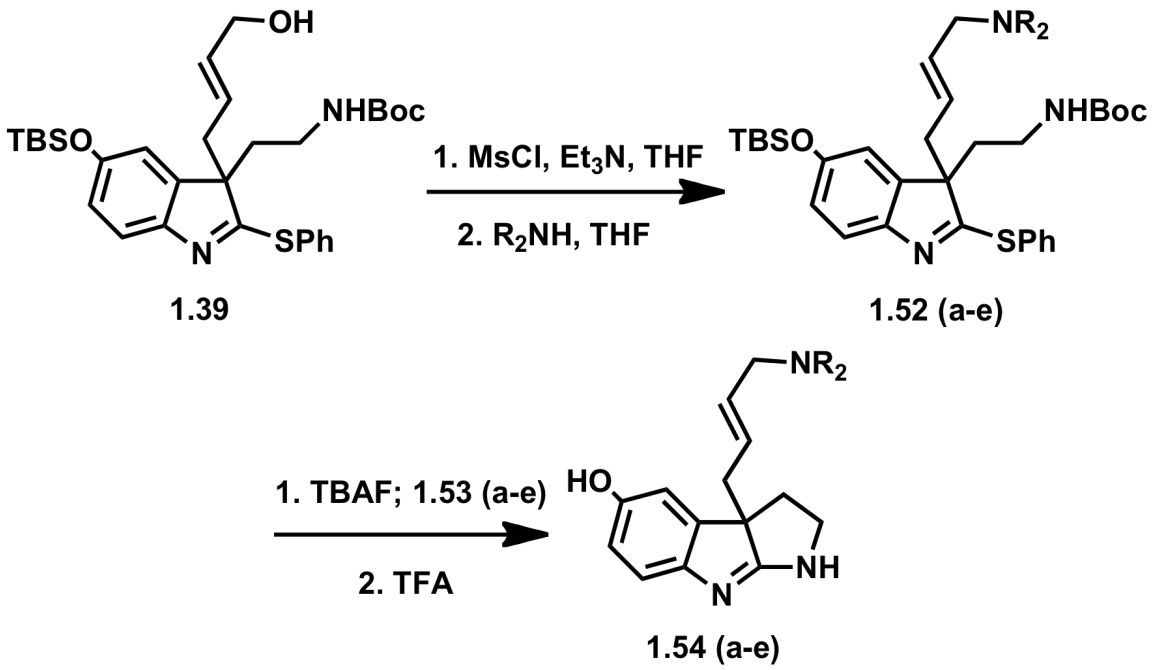
To further expand the reaction scope, we focused our attention on generating a series of allylic tertiary amines from **1.39**. The allylic mesylate from **1.39** was subjected to secondary amines (R_2NH) to give **1.52 (a-e)** (Scheme 1.19). TBAF mediated deprotection of the TBS group provided **1.53 (a-e)**, which on reaction with TFA gave the respective pyrroloindoline compounds **1.54 (a-e)**.

Studies towards phenolic etherification

Reaching our final target compounds required the installation of the phenolic ether side chain. For this purpose we thought to perform the alkylation with a side chain having a tertiary amine in one terminus, and a leaving group on the other end. For instance, Sahu et al. have reported the formation of different basic ether side chains by alkylation of phenol.[19] Reaction of **1.55** with the corresponding ammonium hydrochloride in the presence of K_2CO_3 in acetone provided **1.56** with the respective ether side chain (Scheme 1.20).

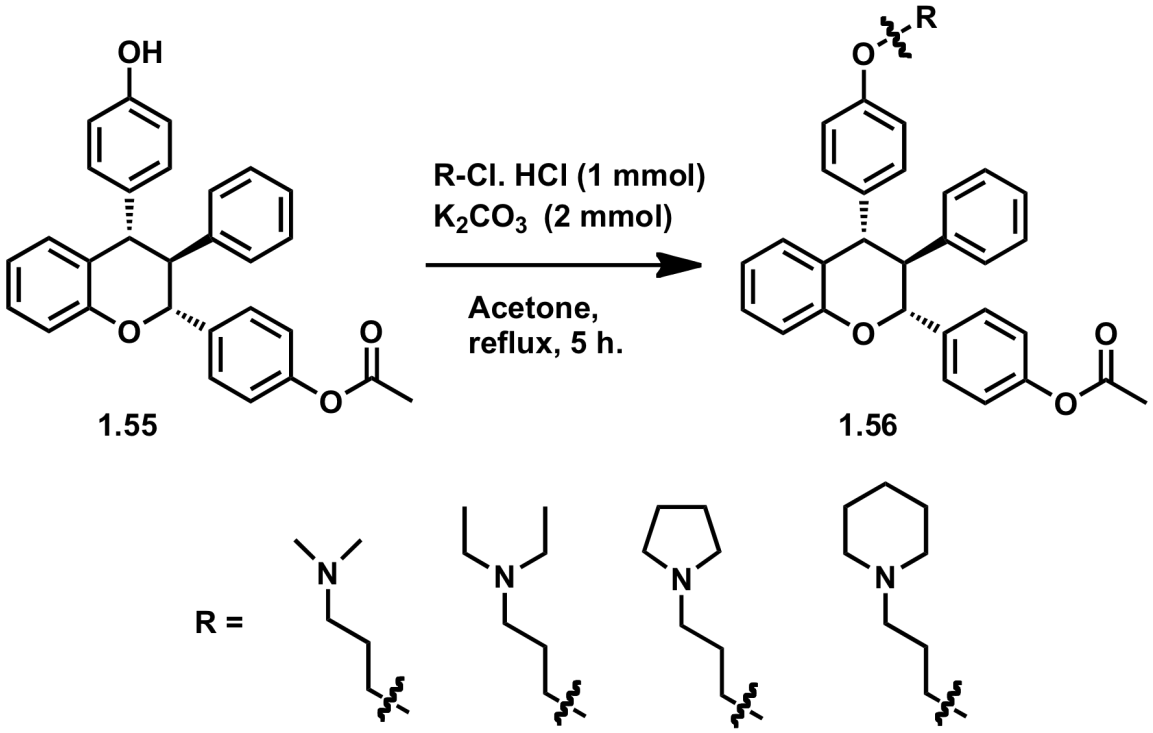
In addition, Mahboobi et al.[20] were able to alkylate the 5-hydroxy indole derivative **1.57** with the alkyl halide side chain **1.58** in the presence of K_2CO_3 in acetone (Scheme 1.21).

However, when we attempted to alkylate **1.50** to give **1.59**, using dimethylaminepropyl chloride (**1.60.a**) or iodide (**1.60.b**) under a number of different conditions including the Finklestein conditions,[21] we were not able to form the phenolic ether bond (Scheme 1.22). Most of the time we were able to recover only the starting material or observed decomposition.

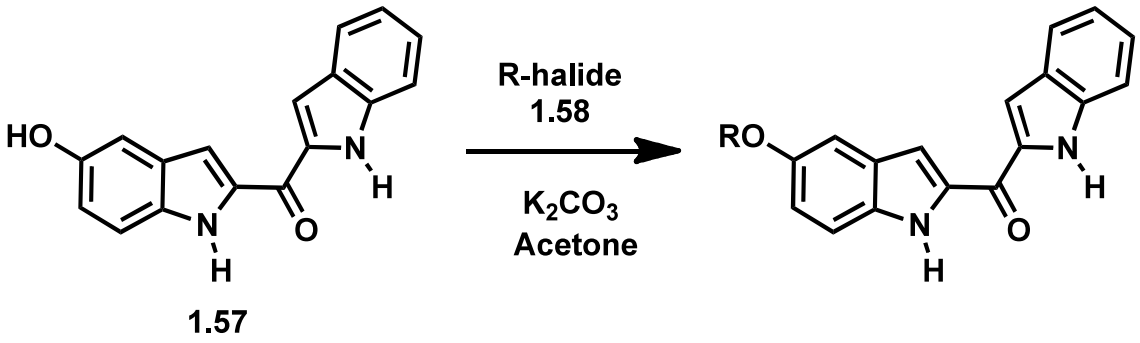


R_2NH	Number	Yield
Diethylamine	**1.52 a**	85–90%
Pyrrolodine	**1.52 b**	85–90%
Piperidine	**1.52 c**	90%
Morpholine	**1.52 d**	85%
N,N,N'-Trimethyl-1,3-propanediamine	**1.52 e**	80%

Scheme 1.19. Attempted synthesis for a series of pyrroloindolines

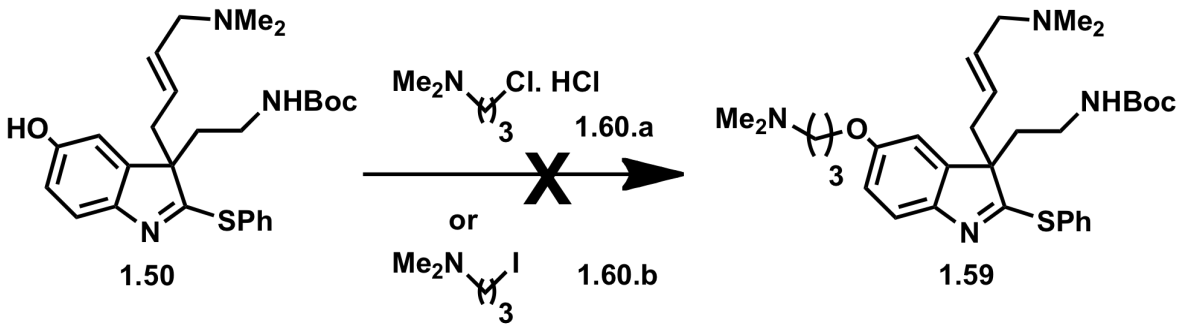


Scheme 1.20. Phenolic alkylation by Sahu et al.



R = $(CH_2)_3$-NMe_2, $(CH_2)_2$-NMe_2, 2-(N-morpholino)-ethyl

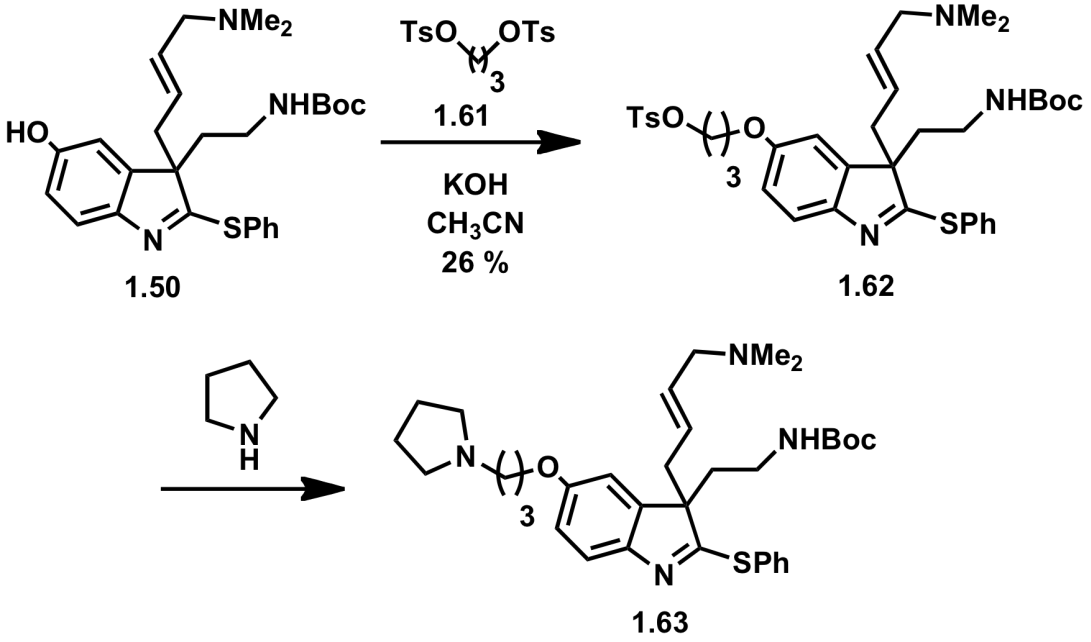
Scheme 1.21. Phenolic alkylation by Mahboobi et al.



Conditions	Result
1.60 a, KOH, CH_3CN, Bu_4NHSO_4	Recovered **1.50**
1.60 a, NaH, DMF, TBAI, 80 ^oC	Decomposition and recovered **1.50**
1.60 a, K_2CO_3, Acetone, reflux	Recovered **1.50**
1.60 a, K_2CO_3, NaI, DMF, 110 ^oC, Microwave	Decomposition and recovered **1.50**
1.60 b, NaH, Acetone	Recovered **1.50**

Scheme 1.22. Some attempted phenolic etherification conditions

Meanwhile, we attempted the alkylation of **1.50** with 1,3-propanediol-di-p-tosylate **1.61** (Scheme 1.23). We were able to isolate the product **1.62**, but only in low yield. Several attempts to enhance the yield were not successful. Although we assumed that the low yields could be either due to dimerization, polymerization, or formation of quaternary ammonium salts, we were not able to isolate any of these. In an attempt to displace the tosyl group of **1.61** in neat pyrrolidine, we observed the possible formation of product **1.62** from the crude ^1H NMR through the disappearance of tosyl group.

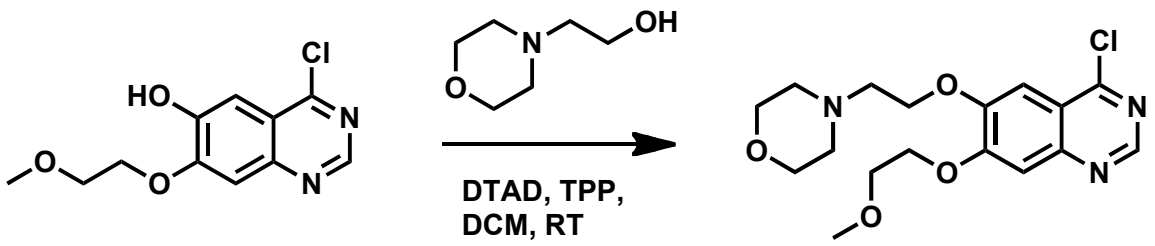


Scheme 1.23. Synthesis of phenolic ether

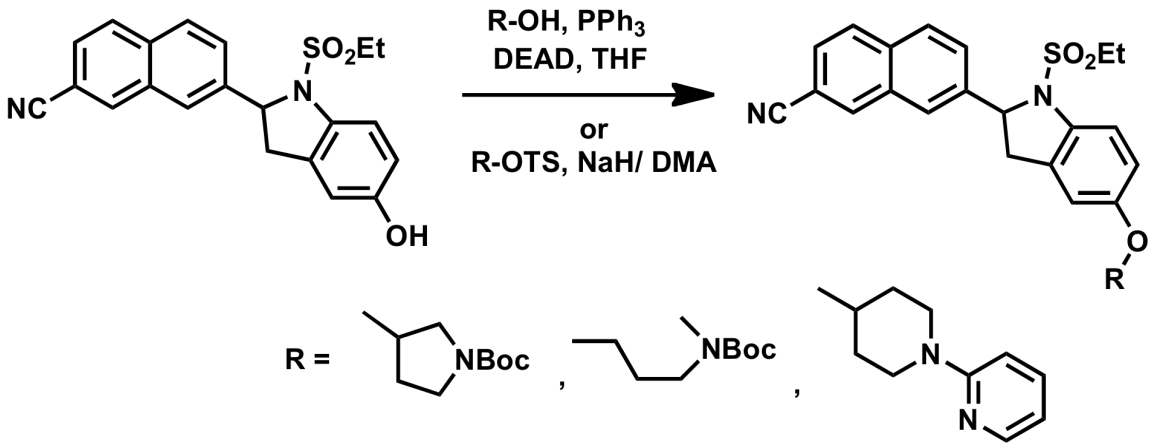
Harris et al., in their studies with quinazoline, have used Mitsunobu conditions for phenolic alkylation with basic side chains (Scheme 1.24).[22] Noguchi et al., in their studies with Factor Xa, have reported Mitsunobu conditions or NaH with a tosylated side chain for the phenolic alkylation (Scheme 1.25).[23]

Our studies, though we used Mitsunobu conditions for the phenolic alkylation, were not successful. When we subjected **1.50** to PPh_3 and DEAD or DIAD and the respective side chain, the starting material **1.50** was recovered (Scheme 1.26).

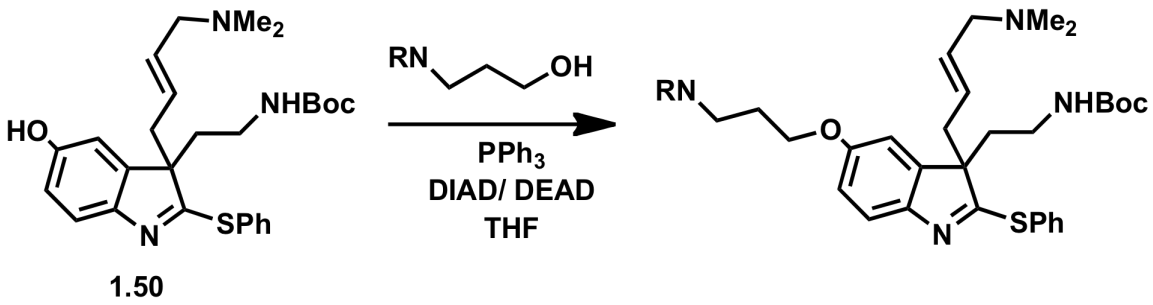
In addition, attempts to tosylate the primary alcohol in **1.64** to give **1.65** were also not successful, for it gave various byproducts (Scheme 1.27). As we expected, this could possibly be due to the polymerization or salt formation.

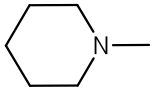


Scheme 1.24. Phenolic etherification under Mitsunobu conditions by Harris et al.

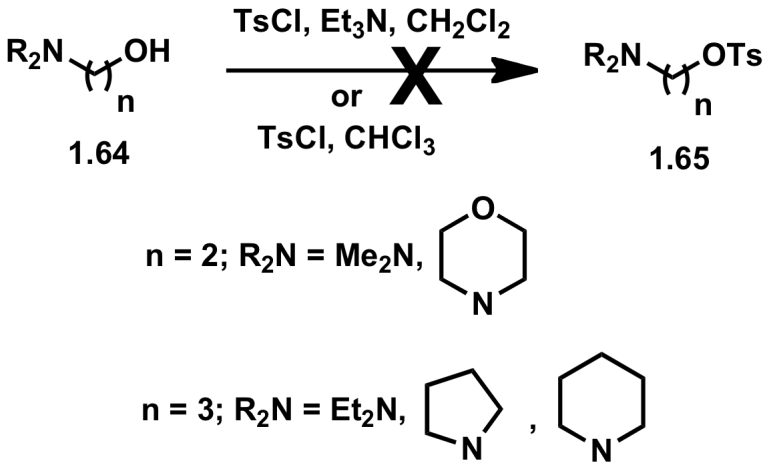


Scheme 1.25. Phenolic etherification by Noguchi et al.



Basic group (RN-)	Conditions
Et_2N-	PPh_3, DIAD, THF, RT
	PPh_3, DIAD, THF, RT
Et_2N-	PPh_3, DEAD, THF, RT, sonication

Scheme 1.26. Attempted Mitsunobu conditions

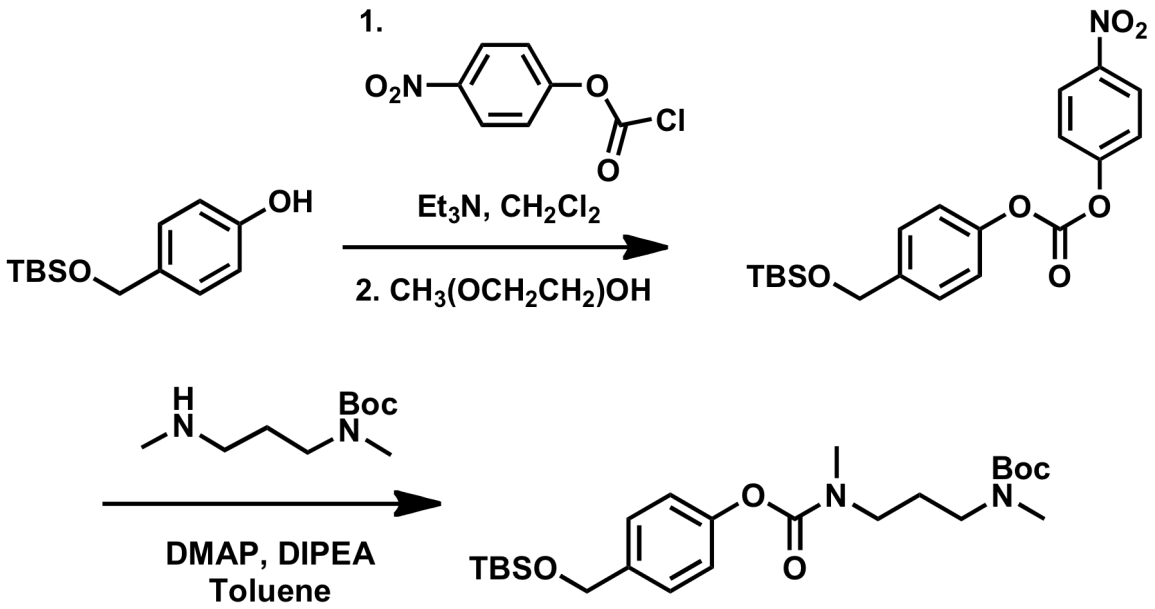


Scheme 1.27. Attempted synthesis for a tosylated side chain

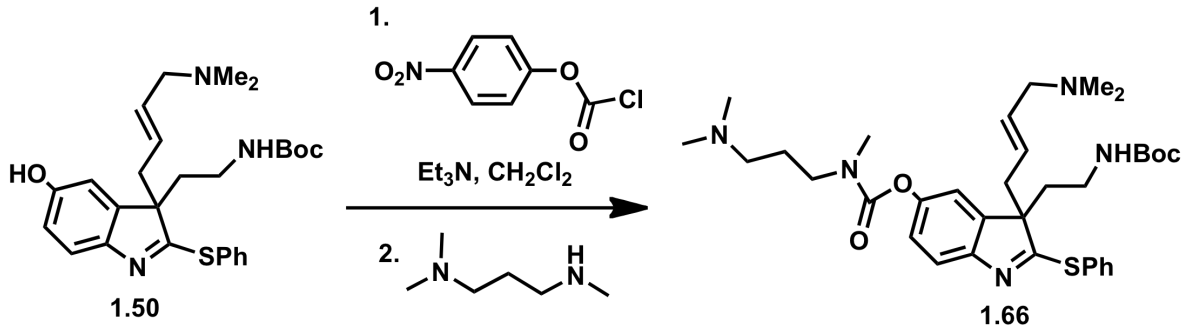
Studies towards phenolic carbamoylation

Carbamate side chains are prevalent in molecules with significant biological activity, such as in cholinesterase inhibitors.[24a,b,c] Gillies et al., in their studies on biodegradable polymers, reported the formation of a carbamate bond between phenol and a secondary amine using p-nitrophenylchloroformate (Scheme 1.28).[25]

With curiosity about how a carbamate side chain would influence the activity of our target compounds, we decided to subject the phenol of **1.50** to carbamoylation conditions (Scheme 1.29). For this purpose, **1.50** was reacted with p-nitrophenylchloroformate and attempts to isolate the product of this reaction were not successful. Therefore, we subjected the reaction mixture immediately to N,N,N'-trimethyl-1,3-propanediamine. However, we were not able to successfully isolate the product **1.66**; instead there were mixtures of byproducts.



Scheme 1.28. Phenolic carbamoylation by Gillies et al.



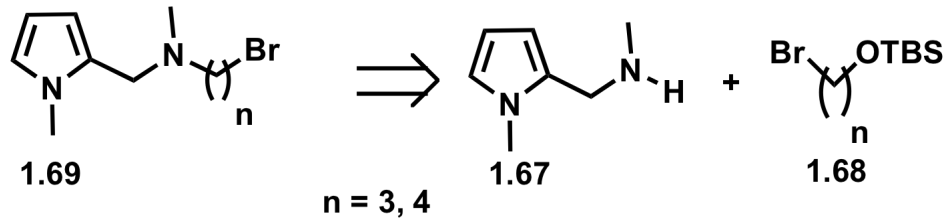
Scheme 1.29. Attempted synthesis for a carbamate side chain

Studies towards synthesis of pyrrole side chain

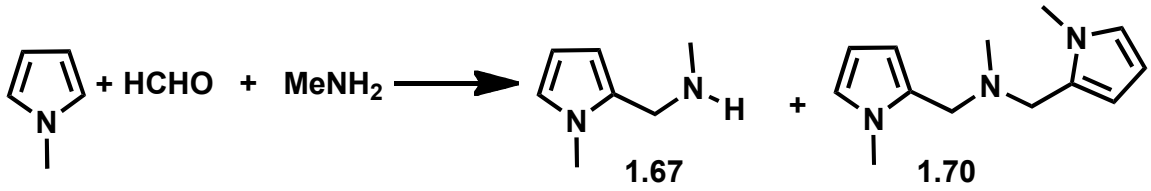
In order to make the basic side chain with the terminal pyrrole group, our initial plan was to react 2-substituted methylpyrrole **1.67** with bromopropanol or bromobutanol **1.68,** to give **1.69** (Scheme 1.30). However, when we attempted to synthesize **1.67**, we were only able to mainly isolate the dimer **1.70** (Scheme 1.31).[26]

Fairhurst et al., in their studies of the Mannich reactions of oxazolidines, reported the synthesis of **1.71.a** from N-methylpyrrole and 3-methyl-1,3-oxazolidine (Scheme 1.32).[27] They also observed the formation of di-substituted product **1.71.b** when the reaction was performed using trichloromethylsilane.

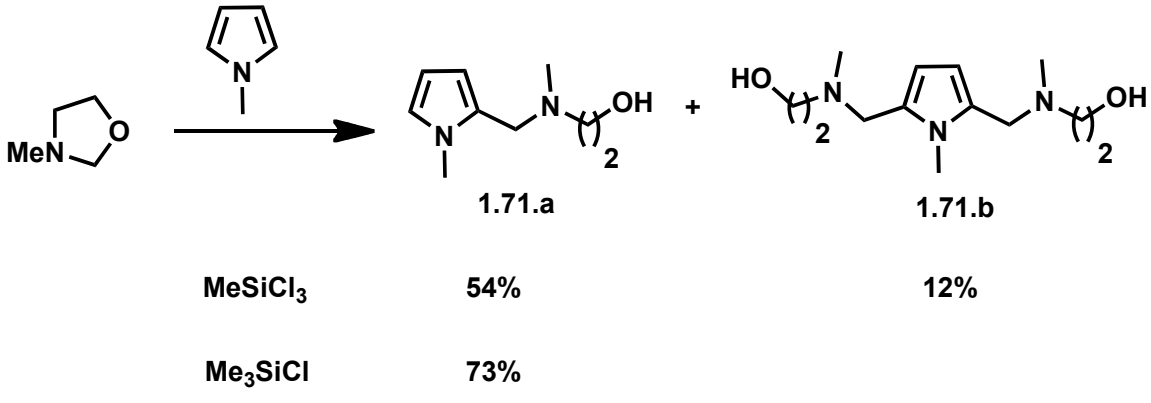
Thus we synthesized secondary amine **1.72** for our studies (Scheme 1.33).[28] The reaction of methylpyrrole with formaldehyde, methylpropanol **1.72**, and TsOH provided **1.73**, but only in low yield (Scheme 1.34). When TsOH was replaced with CSA in MeOH, we observed the deprotection of the TBS ether of **1.73**. TBAF deprotection of the silyl ether in **1.73** and tosylation of the primary alcohol provided the target side chain **1.74**.



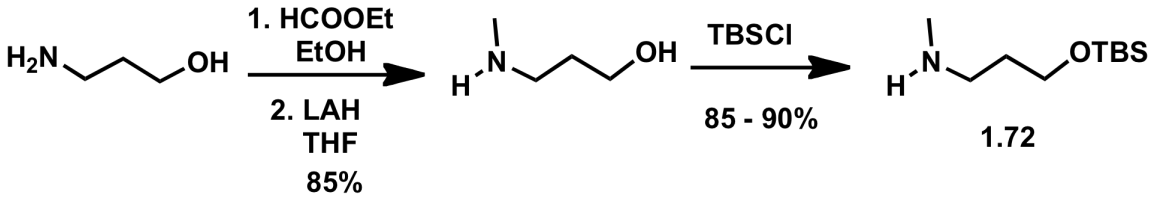
Scheme 1.30. Initial reterosynthetic plan for pyrrole side chain



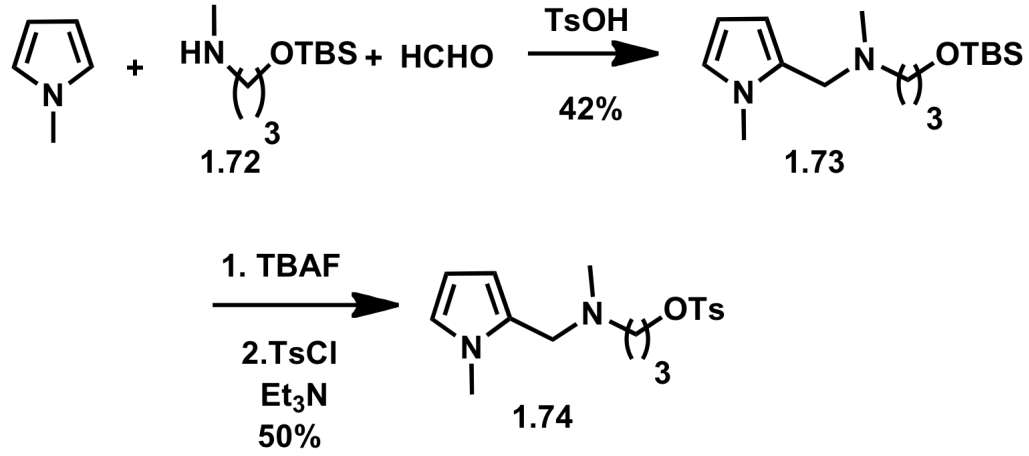
Scheme 1.31. Attempted synthesis for 2-substituted methylpyrrole



Scheme 1.32. Synthesis of 2-substituted methylpyrrole by Fairhurst et al.



Scheme 1.33. Synthesis of methylaminopropanol



Scheme 1.34. Synthesis of pyrrole side chain

Conclusion

Our main goal was to synthesize a series of analogues that would be evaluated for activity against HCV. Based on the preliminary data that we would obtain after screening of **TC 4.121**, and **TC 4.122**, we should be able to further optimize the structure of our inhibitors for enhanced affinity and activity for HCV RNA. It has been observed that compounds based on a benzimidazole or indole core act as non-nucleoside inhibitors of the HCV NS5B polymerase.[3] Thus it would be useful in the future to screen our compounds for HCV RNA IRES, as well as viral enzymes. In addition, screening against

other pathogenic viral IRES would also be valuable for diseases like HIV. Furthermore, as pyrroloindolines are well known for their biological activity,[29] it would be worthwhile to screen our molecules against other medicinally important targets like cancer.

Further efforts are required for installing the phenolic side chain and for optimizing the techniques for isolation of the pyrroloindoline substrates. As studies with these molecules are currently undergoing, it could be possible to realize whether difficulties observed are due to the unpredictable functionality of these compounds, or due to the issues associated with the technique. Also we should note the difficulty associated with purification of these pyrroloindolines. Most of the time, purification was done using preparative silica gel TLC, but it results in a significant decomposition of the product. Purification using neutral or basic alumina was not very successful either. Presumably, purification using reverse phase silica or preparative HPLC would be more efficient.

Current studies from the Rainier group on pyrroloindolines substrates based on **1.75** could further enhance the substrate scope (Figure 1.7).[30] Moreover, future studies with thio-pyranyl/furanyl indolines **1.76** and oxo-pyranyl/furanyl indolines **1.77** would provide us with the prospect of a library of potential inhibitors. In conclusion, we hope our efforts would be successful in achieving our ultimate goal of discovering an effective drug for HCV infection.

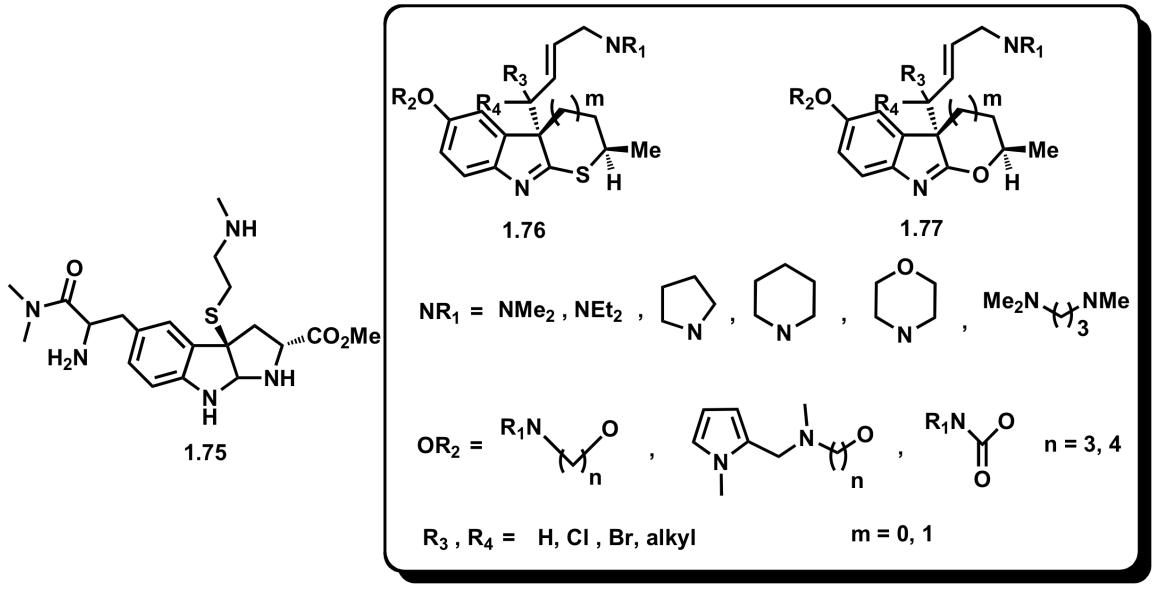


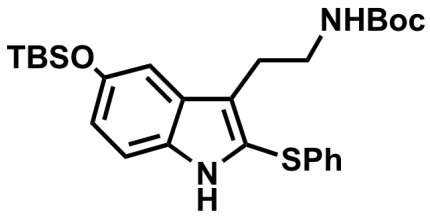
Figure 1.7. Targeted library of potential HCV inhibitors

Experimental Section

Ether and THF were distilled from sodium and benzophenone. CH_2Cl_2, Hexane, Et_3N, MeOH, CH_3CN were distilled from CaH_2. All other reagents were used without further purification unless otherwise stated. Glassware for the reactions was oven dried or flame dried and cooled prior to use. All reactions were run under an atmosphere of nitrogen or argon unless otherwise stated. Thin layer chromatography was performed on Silica gel 60 F_{254} plates eluting with the solvent indicated, visualized by a 254/ 365 nm UV lamp, and stained with solutions p-anisaldehyde, potassium permanganate, Seebach's stain or iodine chamber. Column chromatography was performed with 40–63 μm silica gel, activated neutral alumina or basic alumina. Yields were calculated for material judged homogenous by thin layer chromatography and NMR. Compounds were named using CS ChemBioDraw Ultra 12.0. NMR spectra were acquired on the Varian Unity-

300, VXR 500 or i400 spectrometers. Chemical shifts for ^{1}H NMR spectra are reported in parts per million relative to the signal of residual $CHCl_3$ at 7.27 ppm or the center line of the residual MeOH pentet at 3.31 ppm. Chemicals shifts for ^{13}C NMR spectra are reported in parts per million relative to the center line of the $CDCl_3$ triplet at 77.23 ppm or the center line of the CD_3OD septet at 49.15 ppm. The abbreviations s, d, dd, ddd, dt, t, td, q and m stand for the resonance multiplicity singlet, doublet, doublet of doublet, doublet of doublet of doublet, doublet of triplet, triplet, triplet of doublet, quartet and multiplet respectively. IR spectra were obtained from Nicolet 380 FT-IR spectrometer. Mass spectra were recorded at the Mass Spectrometry facility in the Department of Chemistry of the University of Utah.

Reaction procedures and characterizations of the products



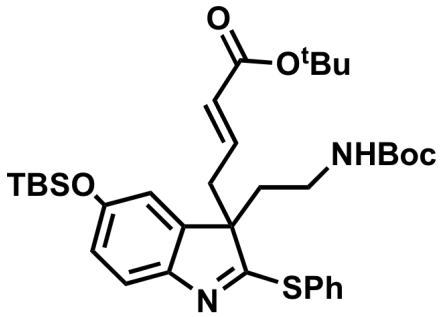
1.17

Synthesis of tert-butyl(2-(5-((tert-butyldimethylsilyl)oxy)-2-(phenylthio)-1H-indol-3-yl)ethyl) carbamate 1.17. To a solution of serotonin hydrochloride (2.527 g, 11.881 mmol) in CH_2Cl_2 (100 ml), Boc_2O (2.593 g, 11.881 mmol) and Et_3N (5 ml, 35.642 mmol) were added at 0 ºC. After stirring at RT overnight the reaction was quenched with saturated NH_4Cl (50 ml). After extraction of the aqueous phase with CH_2Cl_2 (3 x 100 ml), combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated to afford mono Boc protected serotonin **1.33**, which was used forward without further purification.

To the above crude product **1.33** (11.881 mmol) in DMF (10 ml) under an atmosphere of N_2, TBSCl (2.418 g, 16.039 mmol) and imidazole (2.346 g, 34.454 mmol) were added at 0 ºC. After stirring at RT overnight the reaction was quenched with saturated NH_4Cl and extracted the aqueous phase with Et_2O (3 x 100 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated to afford **1.34**, which was used forward without further purification.

To the above crude product **1.34** (11.117 mmol) in CH_2Cl_2 under an atmosphere of N_2, PhSCl (1.896 g, 13.114 mmol) was added dropwise at 0 ºC. After stirring at 0 ºC for 1.5 h, the reaction was quenched with $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The crude material was purified by neutral silica flash column chromatography to afford 4.879 g (88%) of **1.17** as a white foam and some starting material, **1.34**. (R_f = 0.8 in 3: 7 Ethyl acetate: Hexane).

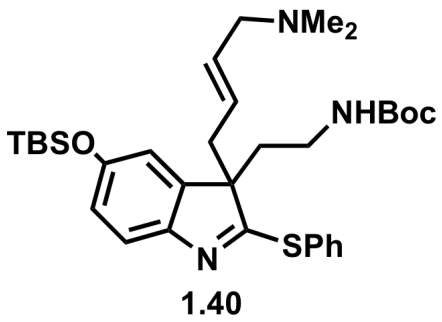
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (broad s, 1 H), 7.26- 7.23 (m, 2 H), 7.19- 7.14 (m, 2 H), 7.09 (d, *J* = 8.1 Hz, 3 H), 6.83 (dd, *J* = 8.7, 2.4 Hz, 1 H), 4.57 (broad s, 1 H), 3.39 (q, *J* = 5.4 Hz, 2 H), 3.01 (t, *J* = 6.4 Hz, 2 H), 1.42 (s, 9 H), 1.04 (s, 9 H), 0.24 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2, 149.6, 137.4, 133, 129.4, 128.7, 126.8, 126.0, 123.3, 120.2, 118.1, 111.7, 108.7, 79.2, 41.2, 28.6, 26.1, 25.5, 18.4, -4.1. IR (neat): 3417 cm^{-1}, 3290 cm^{-1}, 2955 cm^{-1}, 2929 cm^{-1}, 2857 cm^{-1}, 1691 cm^{-1}, 1250 cm^{-1}, 1168 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{27}H_{38}N_2O_3SSi$ (MH+): 499.2, (MNa+): 521.2, (MK+): 537.2; found (MH+): 499.2, (MNa+): 521.2, (MK+): 537.2.



1.38

Synthesis of (E)-tert-butyl 4-(3-(2-((tert-butoxycarbonyl)amino)ethyl)-5-((tertbutyldimethylsilyl)oxy)-2-(phenylthio)-3H-indol-3-yl)but-2-enoate 1.38. To a solution of **1.17** (0.735 g, 1.474 mmol) and $Rh_2(OAc)_4$ (0.033 g, 0.074 mmol) in CH_2Cl_2 (50 ml) at RT under an atmosphere of N_2, was slowly added a solution of freshly prepared vinyl diazo **1.37** (0.588 g, 3.498 mmol) in CH_2Cl_2 (10 ml) from a syringe pump for 10 h. Following the addition reaction was stirred at RT for further 28 h and concentrated. The crude material was purified by neutralized silica flash column chromatography (1:9 EtOAC: Hexane) to afford 0.876 g (93%) of **1.38** as a yellowish brown oil and 0.042 g (5.6 %) of **1.17**. (R_f = 0.5 in 3: 7 Ethyl acetate: Hexane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.66 (m, 2 H), 7.45-7.4 (m, 3 H), 7.26 (dd, *J* = 8.4, 1.4 Hz, 1 H), 6.75 (d, *J* = 8.1, 1 H), 6.72 (s, 1 H), 6.35-6.29 (m, 1 H), 5.75 (d, *J* = 15.8 Hz, 1 H), 4.3 (broad s, 1 H), 2.78-2.74 (m, 3 H), 2.65 (dd, *J* = 13.8, 8.1 Hz, 1 H), 2.21(broad t, 2 H), 1.43 (s, 18 H), 1.00 (s, 9 H), 0.20 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.2, 165.2, 155.8, 153.5, 149.4, 141.8, 140.4, 134.7, 129.5, 128, 127.2,125.1, 120.4, 119.9, 114.4, 80.4, 79.4, 62, 53.8, 41.4, 37.6, 36.7, 28.6, 28.3, 25.9, -4.2. IR (neat): 3359 cm^{-1}, 2957 cm^{-1}, 2930 cm^{-1}, 2858 cm^{-1}, 1712 cm^{-1}, 1463 cm^{-1}, 1157 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{35}H_{50}N_2O_5SSi$ (MH+): 639.3, (MNa+): 661.3, (MK+): 677.3; found (MH+): 639.3, (MNa+): 661.3, (MK+): 677.3.



1.40

Synthesis of (E)-tert-butyl (2-(5-((tert-butyldimethylsilyl)oxy)-3-(4-(dimethylamino)but-2-en-1-yl)-2-(phenylthio)-3H-indol-3-yl)ethyl) carbamate 1.40. To a solution of vinyl ester **1.38** (2.765 g, 4.327 mmol) in CH_2Cl_2 (70 ml), DIBAl-H (8.7 ml, 12.982 mmol) was added dropwise at 0 ºC. After stirring at RT overnight the reaction was quenched with CH_2Cl_2: H_2O: satd. Rochelle salt (12 ml: 12 ml: 12 ml) at 0 °C. After stirring at 0 °C for 1.5 h, the mixture was extracted with CH_2Cl_2 (3 x 50 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated to afford the allylic alcohol **1.39**, which was used forward without further purification.

General procedure A

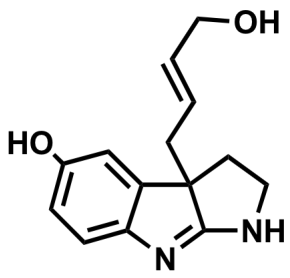
To a solution of allylic alcohol **1.39** (0.637 g, 1.12 mmol) in THF (20 ml), Et_3N (0.47 ml, 3.359 mmol) and MsCl (0.17 ml, 2.239 mmol) were added at 0 °C. After stirring at 0 °C for about 20 min, the reaction was filtered and washed with THF. To the combined mixture of filtrate and washings Me_2NH (11.2 ml, 22.392 mmol) was added at 0 °C. After stirring at RT overnight the reaction was filtered, washed with THF and concentrated. The crude mixture was used forward without further purification or if necessary passed through a neutral alumina column to afford 0.467 g (70%) of **1.40** as a yellowish brown oil. (R_f = 0.45 in 6: 1 CH_2Cl_2: MeOH in silica)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.7-7.67 (m, 2 H), 7.45-7.43 (m, 3 H), 7.21 (d,

J = 9.1 Hz, 1 H), 6.72-6.69 (m, 2 H), 5.6-5.5 (m, 1 H), 5.12-5.02 (m, 1 H), 4.27 (broad s, 1 H), 2.91-2.84 (m, 2 H), 2.77 (q, *J* = 6.7 Hz, 2 H), 2.64 (d, *J* = 7.1 Hz, 2 H), 2.19 (partially obscured t, *J* = 6.1 Hz, 2 H), 2.14 (s, 6 H), 1.4 (s, 9 H), 0.99 (s, 9 H), 0.2 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) 181.6, 155.7, 153.2, 149.4, 142.4, 134.4, 131.9, 129.5, 129.3, 128.2, 126.4, 119.8, 119.3, 114.2, 79.1, 62.6, 61.6, 45.4, 45, 41.8, 37.7, 33.2, 29.8, 28.5, 25.8, -4.3. IR (neat): 2927 cm^{-1}, 2855 cm^{-1}, 1700 cm^{-1}, 1464 cm^{-1}, 1175 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{33}H_{49}N_3O_3SSi$ (MH+): 596.3, (MNa+): 618.3; found (MH+): 596.3, (MNa+): 618.3.

General procedure B

To a solution of allylic alcohol **1.39** (0.021 g, 0.037 mmol) in THF (4 ml) TBAF (0.07 ml, 0.073 mmol) was added at 0 °C. After stirring at 0 °C for about 45 min the reaction was quenched with saturated NH_4Cl and extracted the aqueous phase with EtOAc (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated to afford a crude oil, which was purified using silica gel PTLC (8:2 EtOAC: Hexane) if necessary and used for the next step without further purification.



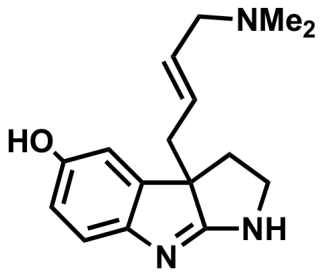
1.49 (TC 4.121)

Synthesis of allylic hydroxy pyrroloindoline 1.49.

General procedure C

To the above crude product (0.022 g, 0.048 mmol) in CH_2Cl_2 (2 ml) under an atmosphere of N_2, TFA (0.5 ml,) was added dropwise at 0 ºC. After the addition reaction was stirred at 0 ºC for 15 min. Then the reaction was slowly warmed to RT, stirred for another 45 min and concentrated. After trituration of the crude reaction mixture with ether, reaction was purified using silica gel PTLC (1: 62: 62 TFA: CH_2Cl_2: MeOH). Product was further purified using silica gel PTLC (1: 53: 53 TFA: CH_2Cl_2: MeOH) to afford 7 mg (85%, two steps from **1.39**) of **TC 4.121** as a yellowish brown oil. (R_f = 0.75 in 1: 62: 62 TFA: CH_2Cl_2: MeOH).

^{1}H NMR (500 MHz, CD_3OD) δ 6.81 (d, *J* = 8.3 Hz, 1 H), 6.72 (d, *J* = 1.1, 1 H), 6.64 (dd, *J* = 8.3, 1.1 Hz, 1 H), 5.54 (td, *J* = 15.1, 5.4 Hz, 1 H), 5.38-5.32 (m, 1 H), 4.2 (dt, *J* = 11.2, 4.9 Hz, 1 H), 3.94 (dd, *J* = 10.7, 8.8 Hz, 1 H), 3.86 (d, *J* = 4.9 Hz, 2 H), 2.57 (dd, *J* = 13.7, 7.3 Hz, 1 H), 2.44 (dd, *J* = 12.2, 4.9 Hz, 1 H), 2.36 (dd, *J* = 13.2, 7.3 Hz, 1 H), 2.28 (dd, *J* = 21, 9.8 Hz, 1 H). ^{13}C NMR (125 MHz, CD_3OD) 163.1, 136.9, 124.3, 121.9, 119.5, 117.2, 116.4, 114.8, 113.6, 63.1, 54.4, 49.4, 40.3, 34.3. IR (neat): 1679 cm^{-1}, 1208 cm^{-1}, 1142 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{14}H_{16}N_2O_2$ (MH+): 245.1; found (MH+): 245.0.

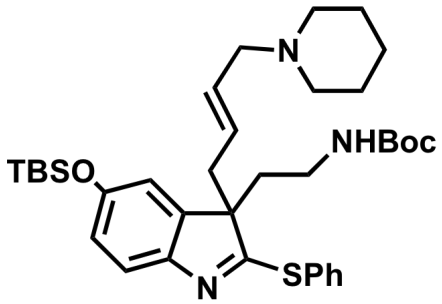


1.51 (TC 4.122)

Synthesis of allylic dimethylamino pyrroloindoline 1.51. Synthesized according to the **General procedure B** using allylic amine **1.40** (0.311 g, 0.522 mmol) and TBAF (1.04 ml, 1.043 mmol) in THF (30 ml). The crude oil was passed through a neutral alumina column (20:1 CH_2Cl_2: MeOH) if necessary and used for the next step without further purification.

Pyrroloindoline synthesis was performed according to the **General procedure C** using above allylic amine (0.009 g, 0.019 mmol) and TFA (0.25 ml) in CH_2Cl_2 (1 ml). Reaction was purified using silica gel PTLC (1: 53: 53 TFA: CH_2Cl_2: MeOH) to afford 4 mg (86%) of **TC 4.122** as a yellowish brown oil. (R_f = 0.7 in 1: 62: 62 TFA: CH_2Cl_2: MeOH)

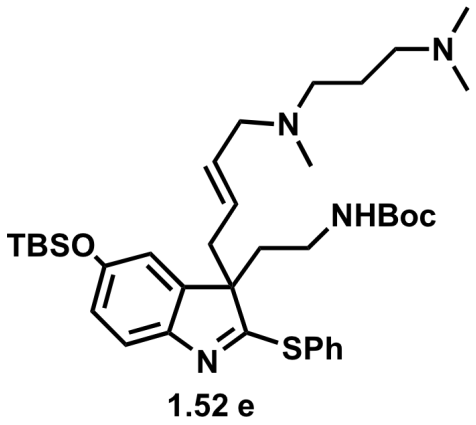
^{1}H NMR (500 MHz, CD_3OD) δ 6.90 (d, *J* = 7.7 Hz, 1 H), 6.83 (s, 1 H), 6.72 (d, *J* = 7.4 Hz, 1 H), 5.74-5.6 (broad m, 1 H), 5.6-5.46 (broad m, 1 H), 4.42-4.3 (broad m, 1 H), 4.14-4.0 (m, 1 H), 3.6-3.53 (m, 2 H), 2.64 (s, 6 H), 2.58-2.4 (broad m, 4 H). ^{13}C NMR (125 MHz, CD_3OD) 180.9, 156.4, 137.2, 123.7, 119.8, 116.3, 116, 113.4, 111.1, 62.3, 59.6, 59.5, 42.5, 40, 36.3. IR (neat): 1680 cm^{-1}, 1209 cm^{-1}, 1140 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{16}H_{21}N_3O$ (MH+): 272.2; found (MH+): 272.1



1.52 c

Synthesis of allylic piperidino indoline 1.52 c. Synthesized according to the **General procedure A** using allylic alcohol **1.39** (0.024 g, 0.043 mmol), Et_3N (0.015 ml, 0.107 mmol) and MsCl (0.008 ml, 0.085 mmol) in THF (4 ml). Subsequently, allylic mesylate was substituted by piperidine (0.08 ml, 0.854 mmol). Neutral alumina flash column chromatography (20: 1 CH_2Cl_2: MeOH) afforded 0.025 g (90%) of **1.52 c** as a yellowish brown oil. (R_f = 0.85 in 6: 1 CH_2Cl_2: MeOH).

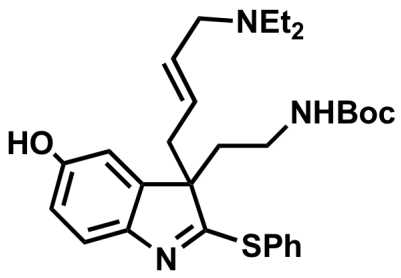
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69-7.66 (m, 2 H), 7.45-7.41 (m, 3 H), 7.21 (d, *J* = 9.1 Hz, 1 H), 6.72 (partially obscured d, *J* = 9.1 Hz, 1 H), 6.69 (s, 1 H), 5.57-5.47 (m, 1 H), 5.04-4.94 (m, 1 H), 4.27 (broad s, 1 H), 2.76 (m, 3 H), 2.69 (partially obscured d, *J* = 8 Hz, 1 H), 2.60 (d, *J* = 7 Hz, 2 H), 2.22-2.04 (broad m, 6 H), 1.52-1.44 (partially obscured m, 6 H), 1.4 (s, 9 H), 0.99 (s, 9 H), 0.2 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.7, 155.8, 153.4, 149.6, 142.5, 134.5, 131.6, 129.5, 129.3, 128.3, 126.6, 120, 119.5, 114.3, 61.4, 60.6, 54.2, 53.6, 42, 38, 36.8, 29.9, 28.6, 25.9, 24.4, 21.3, -4.2. IR (neat): 2931 cm^{-1}, 2850 cm^{-1}, 1713 cm^{-1}, 1463 cm^{-1}, 1274 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{36}H_{53}N_3O_3SSi$ (MH+): 636.4, (MNa+): 658.4; found (MH+): 636.3, (MNa+): 658.4.



1.52 e

Synthesis of indoline 1.52 e. Synthesized according to the **General procedure A** using allylic alcohol **1.39** (0.045 g, 0.079 mmol), Et_3N (0.03 ml, 0.198 mmol) and MsCl (0.01 ml, 0.158 mmol) in THF (8 ml). Subsequently, allylic mesylate was substituted by N,N,N'-trimethyl-1,3-propanediamine (0.23 ml, 1.582 mmol). Silica gel PTLC (6: 1 CH_2Cl_2: MeOH) afforded 0.042 g (80%) of **1.52 e** as a yellowish brown oil. (R_f = 0.8 in 6: 1 CH_2Cl_2: MeOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.66 (m, 2 H), 7.44-7.4 (m, 3 H), 7.2 (d, *J* = 8.8 Hz, 1 H), 6.71 (partially obscured d, *J* = 8.8 Hz, 1 H), 6.69 (s, 1 H), 5.52-5.46 (m, 1 H), 5.05-4.99 (m, 1 H), 4.26 (broad s, 1 H), 2.8-2.68 (m, 4 H), 2.63-2.54 (m, 2 H), 2.21-2.15 (obscured m, 6 H), 2.19 (s, 6 H), 1.97 (s, 3 H), 1.54 (p, *J* = 7.3 Hz, 2 H), 1.39 (s, 9 H), 0.98 (s, 9 H), 0.18 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.7, 155.7, 153.3, 149.5, 142.5, 134.5, 131.8 129.4, 129.3, 128.3, 126.4, 119.9, 119.5, 114.3, 79.3, 59.9, 58.1, 55.5, 48.5, 45.7, 41.9, 41.8, 37.9, 36.7, 35.4, 28.6, 25.9, 25.87, -4.2. IR (neat): 2928 cm^{-1}, 2856 cm^{-1}, 1708 cm^{-1}, 1463 cm^{-1}, 1150 cm^{-1}. LRMS (ESI) m/z calc'd for $C_{37}H_{58}N_4O_3SSi$ (MH+): 667.4, (MNa+): 689.4; found (MH+): 667.4, (MNa+): 689.4.

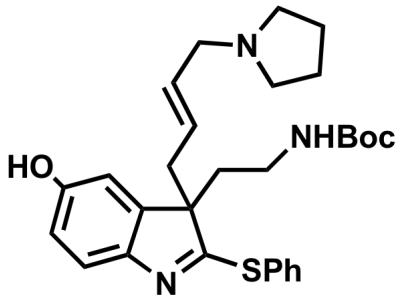


1.53 a

Synthesis of allylic diethylamino indoline 1.53 a. Synthesized according to the **General procedure A** using allylic alcohol **1.39** (0.024 g, 0.043 mmol), Et_3N (0.015 ml, 0.107 mmol) and MsCl (0.008 ml, 0.085 mmol) in THF (4 ml). Subsequently, allylic mesylate was substituted by Et_2NH (0.09 ml, 0.854 mmol). The crude mixture was passed through a neutral alumina column (9: 1 CH_2Cl_2: MeOH) if necessary and used for the next step without further purification.

The TBS group deprotection was performed according to the **General procedure B** using above allylic amine (0.0412 mmol) and TBAF (0.08 ml, 0.082 mmol) in THF (6 ml). Neutral alumina flash column chromatography (9: 1 CH_2Cl_2: MeOH) afforded 0.017 g (81%) of **1.53 a** as a yellowish brown oil. (R_f = 0.4 in 6: 1 CH_2Cl_2: MeOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.7-7.66 (m, 2 H), 7.43-7.4 (m, 3 H), 7.14 (d, *J* = 8.4 Hz, 1 H), 6.93 (partially obscured d, *J* = 1.7 Hz, 1 H), 6.78 (partially obscured dd, *J* = 8.4, 2 Hz, 1 H), 5.68-5.58 (m, 1 H), 5.29-5.2 (m, 1 H), 4.46 (broad s, 1 H), 3.35 (dd, *J* = 13.1, 5.4 Hz, 1 H), 3.08 (dd, *J* = 13.1, 9 Hz, 1 H), 2.86-2.74 (partially obscured m, 2 H), 2.68 (d, *J* = 7 Hz, 2 H), 2.63-2.45 (partially obscured m, 4 H), 2.17 (t, *J* = 6 Hz, 2 H), 1.38 (s, 9 H), 1.1 (t, *J* = 7.4 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 155.9, 155.0, 148.2, 144.5, 142.4, 134.5, 129.5, 129.3, 129.2, 129, 120, 115.3, 110.6, 77.8, 60.6, 54.4, 45.7, 41.7, 38.1, 36.7, 28.6, 14.4. IR (neat): 2955 cm^{-1}, 2918 cm^{-1}, 2849 cm^{-1}, 1273 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{29}H_{39}N_3O_3S$ (MH+): 510.3, (MNa+): 532.3; found (MH+): 510.3, (MNa+): 532.3.

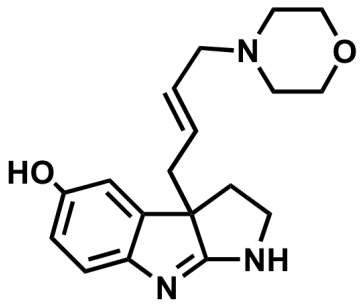


1.53 b

Synthesis of allylic pyrrolidino indoline 1.53 b. Synthesized according to the **General procedure A** using allylic alcohol **1.39** (0.024 g, 0.043 mmol), Et_3N (0.015 ml, 0.107 mmol) and MsCl (0.008 ml, 0.085 mmol) in THF (4 ml). Subsequently, allylic mesylate was substituted by pyrrolidine (0.07 ml, 0.854 mmol). The crude mixture was passed through a neutral alumina column (9: 1 CH_2Cl_2: MeOH) if necessary and used forward without further purification.

The TBS group deprotection was performed according to the **General procedure B** using above allylic amine (0.043 mmol) and TBAF (0.09 ml, 0.085 mmol) in THF (8 ml). Neutral alumina flash column chromatography (9: 1 CH_2Cl_2: MeOH) afforded 0.019 g (86%) of **1.53 b** as a yellowish brown oil. (R_f = 0.4 in 6: 1 CH_2Cl_2: MeOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.67 (m, 2 H), 7.45-7.4 (m, 3 H), 7.17 (d, *J* = 8.3 Hz, 1 H), 6.66 (d, *J* = 2 Hz, 1 H), 6.59 (dd, *J* = 8.3, 2.4 Hz, 1 H), 5.54-5.49 (m, 1 H), 5.12-5.09 (m, 1 H), 4.39 (broad s, 1 H), 2.99 (dd, *J* = 12.2, 5.9 Hz, 1 H), 2.8 (partially obscured dd, *J* = 13.2, 7.8 Hz, 1 H), 2.77-2.73 (broad m, 2 H), 2.55 (d, *J* = 7.3 Hz, 2 H), 2.28-2.23 (m, 4 H), 2.14 (t, *J* = 7 Hz, 2 H), 1.69 (broad s, 4 H) 1.4 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 155, 148.1, 142.5, 134.5, 130.3 (broad), 129.5, 129.3, 128.3 (broad), 120.1, 115.2, 110.6, 57.6, 53.4, 41.7, 37.9, 36.7, 33.9, 28.6, 23.4. IR (neat): 2956 cm^{-1}, 2917 cm^{-1}, 2849 cm^{-1}, 1707 cm^{-1}, 1462 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{29}H_{37}N_3O_3S$ (MH+): 508.3; found (MH+): 508.3.



1.54 d

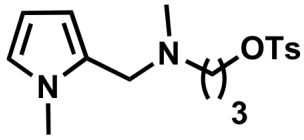
Synthesis of allylic morpholino pyrroloindoline 1.54 d. Synthesized according to the **General procedure A** using allylic alcohol **1.39** (0.024 g, 0.043 mmol), Et_3N (0.015 ml, 0.107 mmol) and MsCl (0.008 ml, 0.085 mmol) in THF (4 ml). Subsequently, allylic mesylate was substituted by morpholine (0.07 ml, 0.854 mmol). The crude mixture was passed through a neutral alumina column (9: 1 CH_2Cl_2: MeOH) if necessary and used forward without further purification.

The TBS group deprotection was performed according to the **General procedure B** using the above allylic amine (0.043 mmol) and TBAF (0.09 ml, 0.085 mmol) in THF (8 ml). The crude oil was passed through a neutral alumina column (9: 1 CH_2Cl_2: MeOH) if necessary and used for the next step without further purification.

Pyrroloindoline synthesis was performed according to the **General procedure C** using above allylic amine (0.036 mmol) and TFA (0.3 ml) in CH_2Cl_2 (3 ml). Reaction was purified using silica gel PTLC (4: 1 CH_2Cl_2: MeOH) to afford 8 mg (75%) of **1.54 d** as a yellowish brown oil. (R_f = 0.2 in 4: 1 CH_2Cl_2: MeOH).

^{1}H NMR (500 MHz, CD_3OD) δ 6.71 (d, *J* = 8.2 Hz, 1 H), 6.65 (d, *J* = 2.2 Hz, 1 H), 6.56 (dd, *J* = 8.2, 2.5 Hz, 1 H), 5.34-5.31 (m, 2 H), 4.04-3.99 (m, 1 H), 3.81 (dd, *J* = 11.5, 8.2 Hz, 1 H), 3.62 (dd, *J* = 4.7, 4.4 Hz, 4 H), 2.89 (dd, *J* = 12.4, 3.3 Hz, 1 H), 2.72 (dd, *J* = 12.9, 6 Hz, 1 H), 2.54- 2.46 (m, 2 H), 2.28 (partially obscured dd, *J* = 12.1, 5 Hz, 1 H), 2.20 (partially obscured dd, *J* = 4.7, 4.4 Hz, 4 H), 2.1 (dd, *J* = 20.6, 10.4 Hz, 1 H).

IR (neat): 2922 cm^{-1}, 2852 cm^{-1}, 1676 cm^{-1}, 1208 cm^{-1}, 1137 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{18}H_{23}N_3O_2$ (MH+): 314.2; found (MH+): 314.2.



1.74

Synthesis of pyrrole side chain 1.74. To a solution of HCHO (0.01 ml, 0.096 mmol), amine **1.72** (0.019 g, 0.096 mmol) and $TsOH.H_2O$ (0.002 g, 0.008 mmol) was added and stirred for 30 min. The mixture was slowly added into methylpyrrole (0.01 ml, 0.08 mmol) at 0 ^oC for 45 min. After stirring at RT overnight, the reaction was quenched with saturated $NaHCO_3$ and extracted the aqueous phase with CH_2Cl_2 (3 x 15 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The crude material was purified by neutralized silica flash column chromatography (1:9 EtOAC: Hexane) to afford **1.73**.

To a solution of **1.73** (0.006 g, 0.021 mmol) in THF (1 ml), TBAF (0.04 ml, 0.041mmol) was added at 0 ^oC. After stirring at 0 ^oC for 1 h the reaction was quenched with saturated NH_4Cl and extracted the aqueous phase with CH_2Cl_2 (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The crude material was purified using silica gel PTLC (6:4 EtOAC: Hexane).

To the above primary alcohol (0.003 g, 0.018 mmol) in CH_2Cl_2 (1 ml), TsCl (0.004 g, 0.021 mmol), Et_3N (0.01 ml, 0.07 mmol) and catalytic amount of DMAP was added at 0 ^oC. After stirring at RT overnight the reaction was quenched with saturated NH_4Cl and extracted the aqueous phase with CH_2Cl_2 (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The crude material was purified using silica gel PTLC (3:7 EtOAC: Hexane) to afford 3 mg (53%) of **1.74** as

a brownish oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.4 Hz, 2 H), 7.35 (d, *J* = 8.4 Hz, 2 H), 6.58 (s, 1 H), 6.02 (dd, *J* = 3.4, 2.7 Hz, 1 H), 5.94 (s, 1 H), 4.07 (t, *J* = 6.1, 6.7 Hz, 2 H), 3.56 (s, 3 H), 3.36 (s, 2 H), 2.47 (s, 3 H), 2.38 (t, *J* = 6.7 Hz, 2 H), 2.08 (s, 3 H), 1.79 (tt, *J* = 7.1, 6.7 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.8, 130.1, 129.9, 128.1, 128.0, 122.7, 109.7, 106.4, 69.1, 54.4, 52.9, 41.7, 31.1, 27.0, 21.8. IR (neat): 3400 cm^{-1}, 2924 cm^{-1}, 2854 cm^{-1}, 1192 cm^{-1}. LRMS (ESI) m/z calc'd for $C_{17}H_{24}N_2O_3S$ (MNa+): 359.1, (MK+): 375.1; found (MNa+): 359.1, (MK+): 375.1.

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CHAPTER 2

STUDIES TOWARDS THE EPIDITHIODIKETOPIPERAZINE ALKYLATION IN CHAETOMIN

Introduction

2,5-Diketopiperazines (DKPs) are cyclic dipeptides ubiquitous in nature, and are attributed with a multitude of appealing biological activities, such as anti cancer activity, neuroprotective effects, antihelmintic properties, tryptase inhibition and oxytocin receptor antagonism.[1] Enormous efforts have been reported for the synthesis of these alkaloids due to their fascinating architecture, coupled with their interesting biological activity.[2] Among the DKP targets are the fungal secondary metabolites, called ETPs (epipolythiodioxopiperazines), which contain sulfide bridges (di, tri or tetra) across the 3,6- positions of the DKPs.[3] It is believed that the striking biological activities of these molecules are due to their sulfide bridges, which can directly conjugate to cysteine residues or generate reactive oxygen species by redox cycling. Most natural ETPs are derived from the amino acid tryptophan, and contain an ETP ring fused to a cyclotryptamine. [4]

Kishi's total synthesis of fungal metabolite gliotoxin (**2.1**) is a milestone in the synthetic efforts towards natural products containing ETPs (Figure 2.1).[5] Sporidesmin A

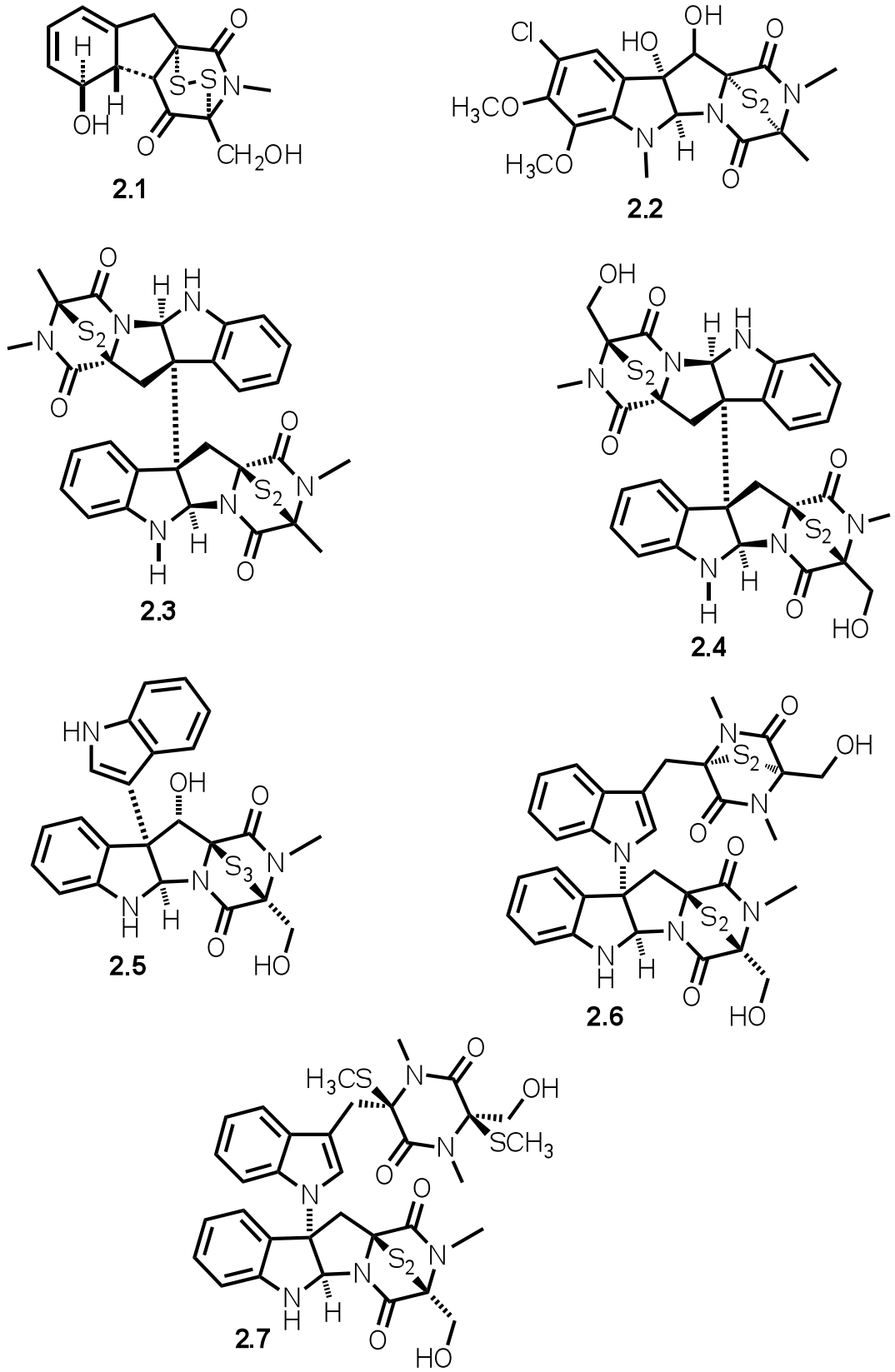


Figure 2.1. Some structurally and functionally important epithiodiketopiperazines

(**2.2**),[6] cytotoxic, dimeric ETP (+)-11,11'-dideoxyverticillin A (**2.3**),[7] and dimeric (+)-chaetocin (**2.4**),[8] are among the ETP natural products that have been synthesized. Cytotoxic T988 A (**2.5**),[9] chaetomin (**2.6**), and chaetocochins C (**2.7**),[10] are among the ETP natural products that have not been synthesized.

Chaetomin was first isolated by Waksman and Bugie[11] from a strain of *Chaetomium cochliodes*, and was found to possess antibiotic activity[12] and immunomodulatory activity.[13] Recently, chaetomin spurred interest among scientists by exhibiting anti cancer activity by inhibiting the hypoxia inducible factor 1 α (HIF-1 α), binding to the target protein p300/CBP.[14] As hypoxia enhances radioresistance in tumors and mediates resistance to chemotherapy, it was found that chaetomin influences the radiosensitivity of hypoxic cells in vitro.[15]

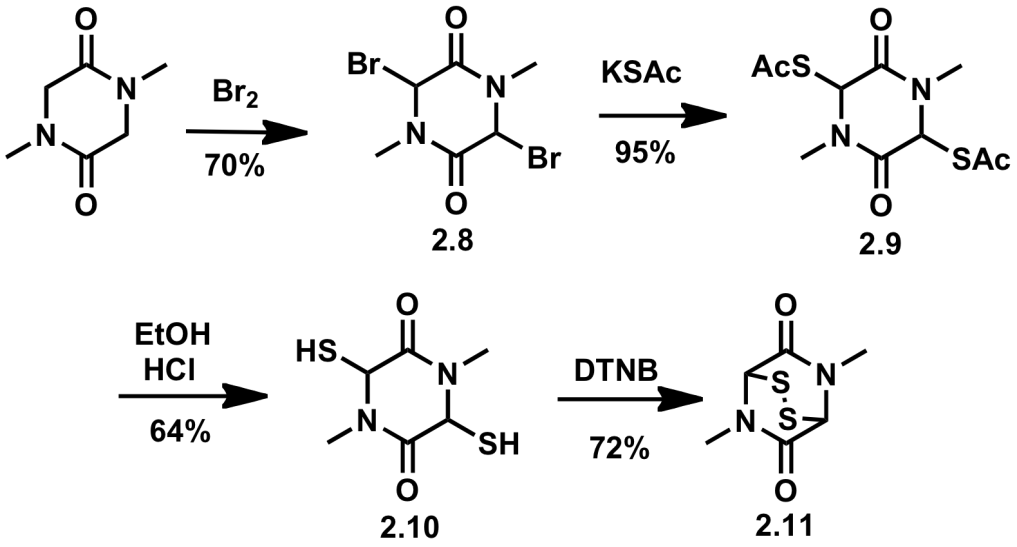
Hypoxia, the state of reduced oxygen levels, is characteristic of tumors. Adaptations to hypoxic conditions are mediated by a transcription factor HIF-1; thus HIF-1 has become an attractive target in anticancer therapy. HIF-1 is a heterodimeric protein comprising of HIF-1α and HIF-1 β subunits. HIF-1 α degrades rapidly under normoxic conditions and stabilizes under hypoxic conditions.[16] Under hypoxic conditions, the HIF-1 α subunit binds to the transcriptional coactivator p300/CBP; it subsequently dimerizes by binding to the HIF-1 β subunit. Thereby, the dimer activates the hypoxia-inducible genes, such as genes that control angiogenesis, energy metabolism, and erythropoiesis.[17] Chaetomin disrupts the structure of the p300 and precludes its interaction with HIF-1 α, thereby interfering with the survival of tumors under hypoxic conditions. Thus, chaetomin's conspicuous biological role and its intricate heterodimeric architecture have made it an attractive synthetic target.

Background

The highly reactive disulfide bridge is one of the major challenges in the synthesis of epidithiodiketopiperazine natural products. The first synthesis of an ETP moiety was reported by Trown in 1968 (Scheme 2.1).[18] Bromination of the sarcosine anhydride afforded the 3,6-dibromide **2.8** in 70% yield. Nucleophilic displacement of bromine with potassium thioacetate afforded the 3,6-dithioacetate **2.9** in 95% yield. Hydrolysis of **2.9** in ethanolic HCl provided the dimercaptan **2.10**, subsequent oxidation with Ellman's reagent gave the disulfide **2.11** in 72% yield. Disulfide **2.11** exhibited antiviral activity and desulfurization resulted in a loss of biological activity, which signifies the fact that the active center of these ETP natural products is the epidithiapiperazinedione moiety.

Inspired by the chaetocin's (**2.4**) and chaetomin's (**2.6**) biological activity, Block et al. designed a small molecule, dimeric ETP, which is an inhibitor of the hypoxia-inducible transcription factor complex.[19] They observed disulfide dimer **2.12** to have significant activity compared to the control **2.13**, which lacks the disulfide bridge (Figure 2.2). This loss of activity in **2.13** further emphasizes the importance of ETP moiety for the biological role.

The highly labile nature of the disulfide bridge under reductive, basic, and acidic conditions is a major concern in the total synthesis of ETP natural products. Therefore, Kishi and coworkers, in their synthesis of ETP natural products, protected the dithiol **2.10** as the p-anisaldehydedithioacetal **2.14**, by reaction with p-anisaldehyde and $BF_3{\cdot}OEt_2$ (Scheme 2.2). The thioacetal is stable under acidic, basic or reductive conditions and can be smoothly cleaved to the disulfide **2.11** with m-CPBA and acid (such as $BF_3{\cdot}OEt_2$, $BC1_3$, H_2SO_4 or $HClO_4$).



Scheme 2.1. Trown's synthesis of epidithiodiketopiperazine

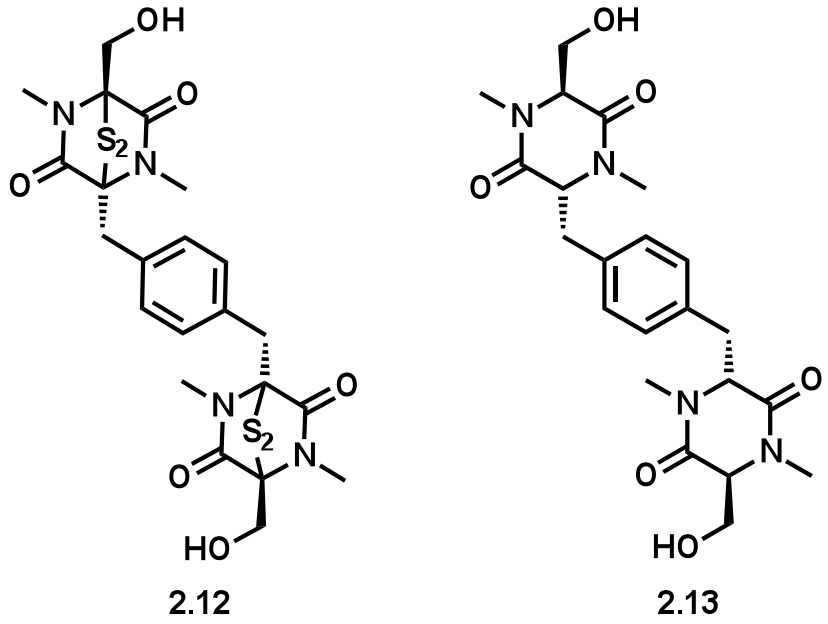
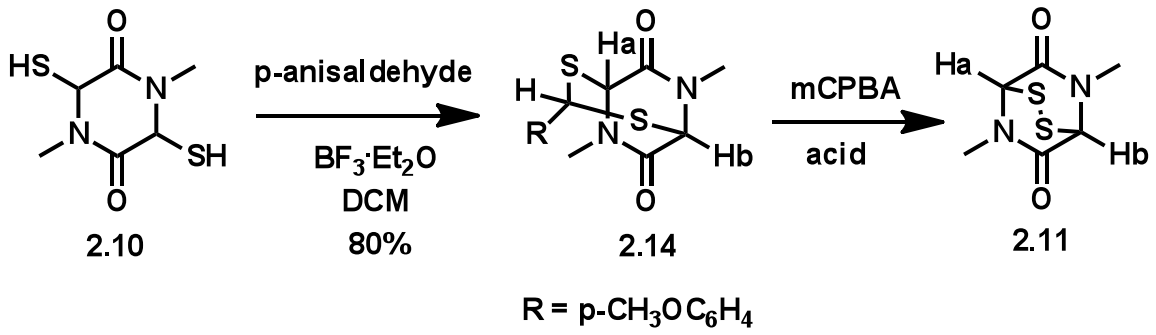


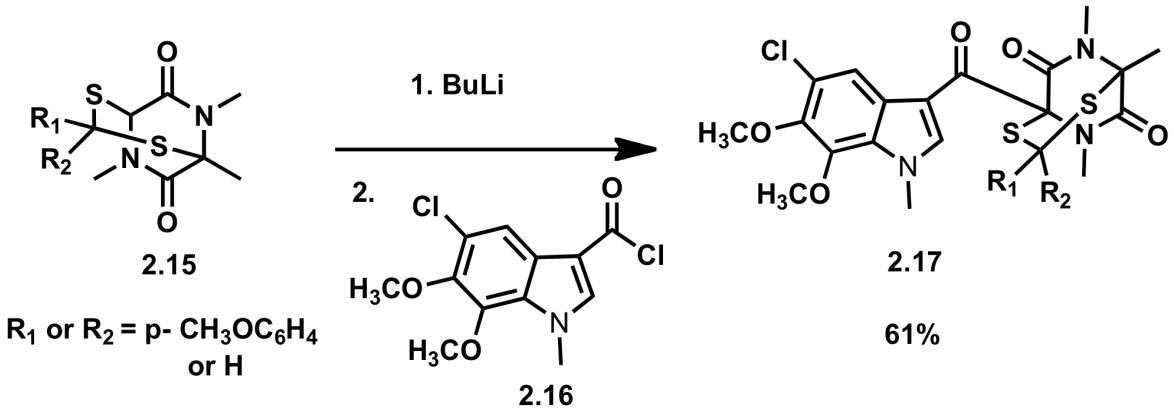
Figure 2.2. Molecules designed by Block et al. for HIF inhibition



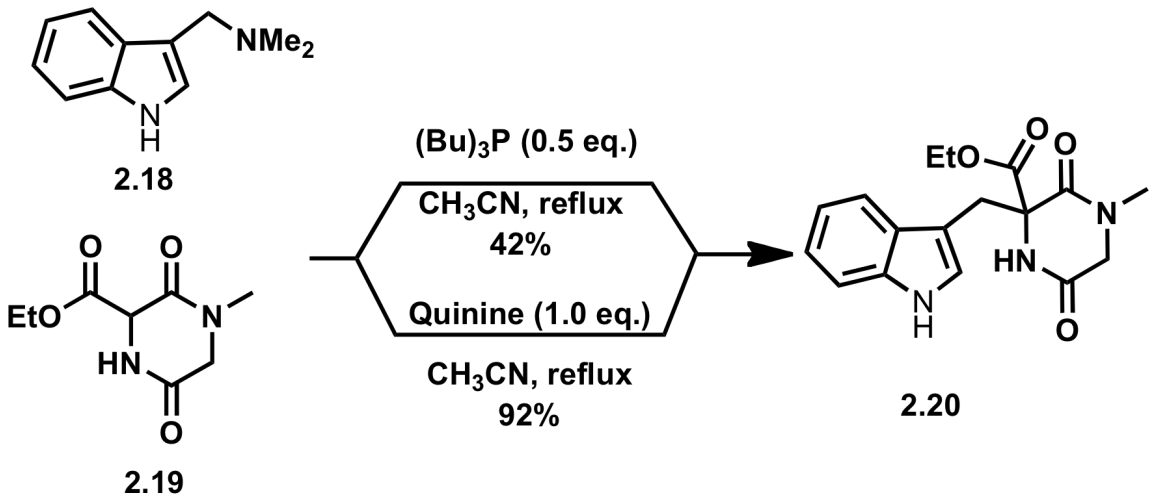
Scheme 2.2. Kishi's synthesis of dithioacetal

Interestingly, Kishi and coworkers observed an acidity difference in the bridgehead protons Ha and Hb, which enables them to regiospecifically functionalize the bridgehead positions. The bridgehead carbanion was generated using alkyllithium reagents, which was immediately quenched with an alkylating or acylating agent.[20]

The indole-diketopiperazine bridge is an important structural feature of the northern hemisphere of chaetomin **2.6**. Several attempts have been reported in the literature on alkylation of DKP with an indole moiety. For instance, in the total synthesis of sporidesmin A,[6] bridgehead carbanion of **2.15** was generated using butyllithium, which was then acylated with indole acyl chloride **2.16** to give **2.17** (Scheme 2.3). Furthermore, Kametani and Somei reported coupling of gramine **2.18** with the nucleophilic DKP substrate **2.19** in the presence of trialkylphosphine to give **2.20** in 42% yield (Scheme 2.4). During the course of our synthesis, Dubey and Olenyuk reported their attempts to improve the efficiency and yields of the above reaction and were able to obtain higher yields by replacing trialkylphosphine with quinine.[21] Outlined here in the second chapter of this thesis are studies directed towards the synthesis of indole-diketopiperazines.



Scheme 2.3. Indole-diketopiperazine bridge formation in sporidesmin A



Scheme 2.4. Alkylation of diketopiperazine with gramine

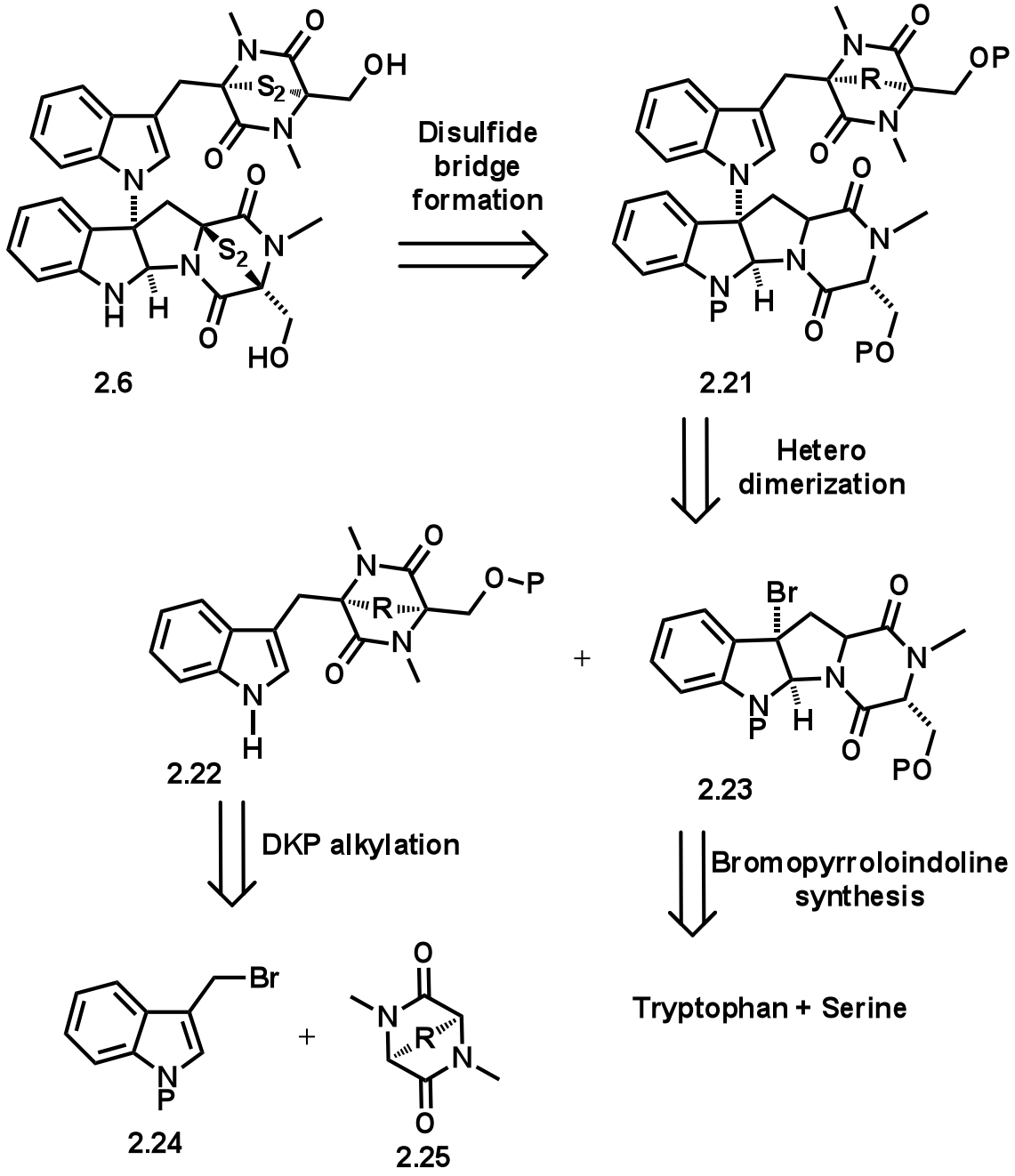
Reterosynthesis

A convergent reterosynthetic approach towards chaetomin is proposed here (Scheme 2.5). It was envisioned that chaetomin **2.6** would arise from the final disulfide bridge installation in **2.21**. The chaetomin precursor **2.21** would come from the coupling of advanced intermediates **2.22** and **2.23**, using the C(3)-N(1') heterodimeric indoline synthesis strategy developed by our group.[22] The **2.22** would arise from the alkylation of the skatole derivative **2.24** with DKP **2.25** and subsequent primary alcohol installation. The DKP synthesis between tryptophan and serine and the subsequent bromocyclization would provide the bromopyrroloindoline **2.23**.

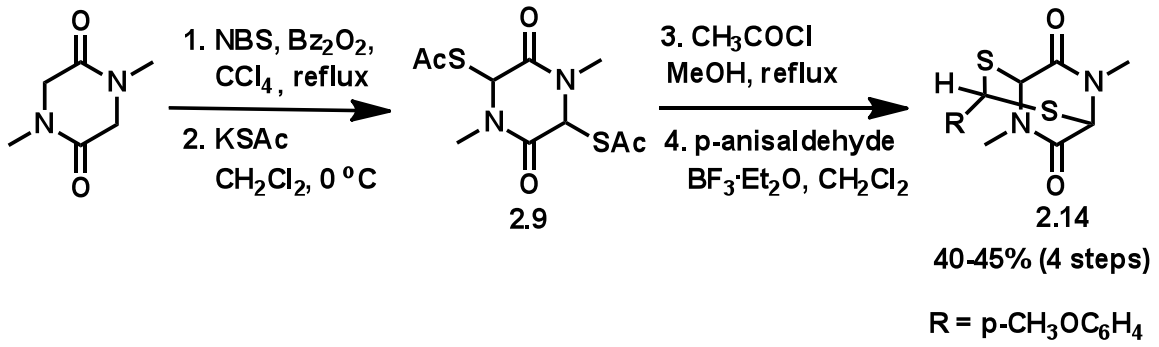
Results and Discussion

Initially, we synthesized diketopiperazine derivative **2.14** from sarcosine anhydride (Scheme 2.6).[18,20] Sarcosine anhydride was reacted with NBS to give the corresponding dibrominated product, which on reaction with KSAc provided the dithioacetate product **2.9**. Subsequent hydrolysis of the acetate ester in acidic MeOH provided the dithiol compound, which was protected with p-anisaldehyde in $BF_3{\cdot}OEt_2$, to give p-anisaldehydedithioacetal **2.14** in 40-45% yield over four steps. With **2.14** in hand, the next step was to investigate the coupling of **2.14** with the indole moiety.

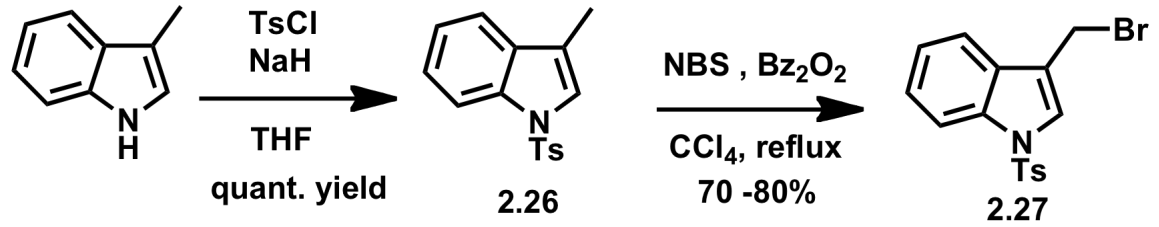
Towards this endeavor, tosylation of skatole provided **2.26**, which was brominated in the presence of NBS and Bz_2O_2 to give **2.27** (Scheme 2.7).[23] In order to form the indole-diketopiperazine bridge, the bridgehead carbanion of **2.14** was generated with LHMDS in THF at -78 ^oC (Scheme 2.8). Subsequent quenching of the carbanion with **2.27** provided the alkylated ETP product **2.28** in 25% yield with isolated **2.14**.



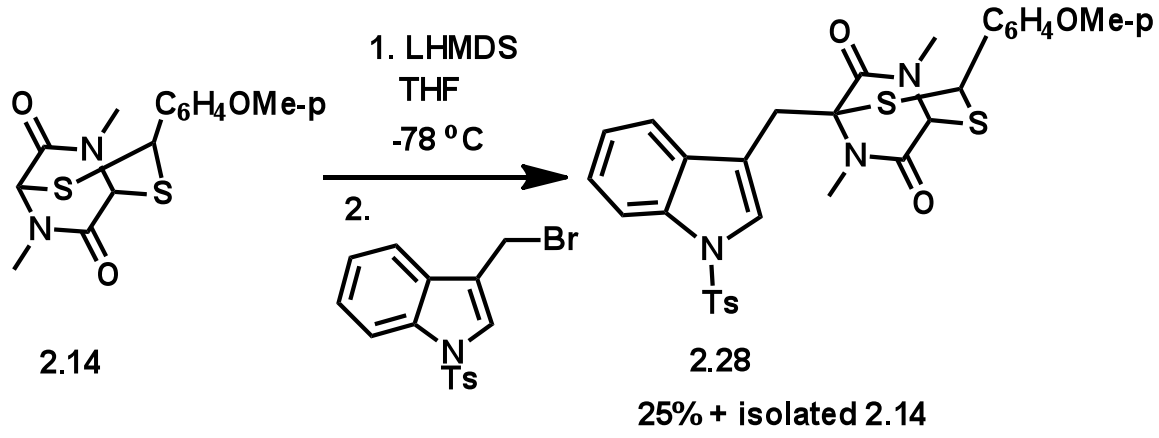
Scheme 2.5. Reterosynthesis of chaetomin



Scheme 2.6. Synthesis of p-anisaldehydedithioacetal



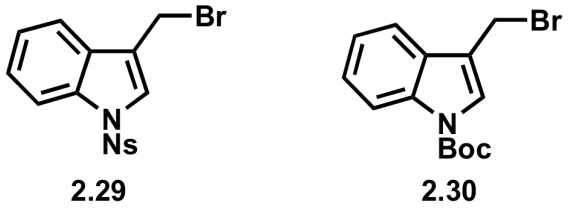
Scheme 2.7. Synthesis of brominated N-tosyl skatole



Scheme 2.8. Synthesis of indole-diketopiperazine bridge

The tosyl group in **2.28** needed to be deprotected prior to the C(3)-N(1') heterodimeric coupling with bromopyrroloindoline **2.23**. Although the tosyl group is known to be cleaved under much harsher reductive conditions, we were much concerned about the sensitivity of thioacetal in **2.28** under these harsher conditions. There are several reports of the removal of the tosyl group under milder conditions, such as in KOH/ H_2O/ THF,[24a] and Cs_2CO_3/ MeOH/ THF.[24b] Attempts to deprotect the tosyl group in **2.28** using KOH, NaOH, and NaH in MeOH proved to be fruitless, resulting in recovery of starting material. Meanwhile, in trying to optimize the conditions for the detosylation, we intended for the synthesis of the brominated skatole with a different protecting group, which could be cleaved under much milder conditions.

Thus our first choice was to have a nosyl group instead of tosyl, so that it could be cleaved under much milder conditions, such as K_2CO_3, Cs_2CO_3 or PhSH. However, following the same procedure as in **2.27**, attempts to brominate nosylated skatole to give **2.29** were not very successful (Figure 2.3). Therefore, we decided to protect skatole with a Boc group, planning on cleaving it with TFA at a later stage. It is reported that the DKP thioacetals are usually stable to acidic conditions.[20] This stability was proved again when we stirred **2.28** in TFA overnight, wherein we were able to still isolate the **2.28** unaffected. Thus we synthesized Boc protected brominated skatole **2.30**,[25] and focused on subsequent alkylation with thioacetal protected DKP **2.14**. It should be noted that care should be involved in handling these brominated skatole moieties. They are highly unstable, and in a matter of hours at RT lead to a debromo product, which cannot induce the subsequent alkylation reaction.



2.29 2.30

Figure 2.3. Brominated skatole derivatives

Conclusion

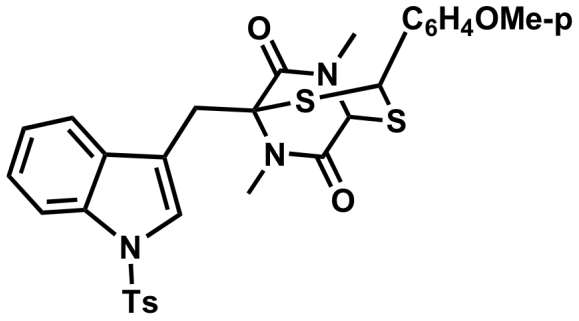
To our knowledge, the above method provides a rapid and convergent entry into the northern hemisphere of chaetomin. However, more work needs to be done to optimize the alkylation reaction to gain higher yields, and to find suitable conditions for detosylation. Studies aimed at accomplishing these goals are underway.

Experimental Section

THF was distilled from sodium and benzophenone. All other reagents were used without further purification unless otherwise stated. Glassware for the reactions was oven dried or flame dried and cooled prior to use. All reactions were run under an atmosphere of nitrogen or argon unless otherwise stated. Yields were calculated for material judged homogenous by thin layer chromatography and NMR. Thin layer chromatography was performed on Silica gel 60 F_{254} plates eluting with the solvent indicated, visualized by a 254/ 365 nm UV lamp, and stained with solutions of p-anisaldehyde, potassium permanganate or iodine chamber. Column chromatography was performed with 40–63 μm silica gel. NMR spectra were acquired on the i400 spectrometer. Chemical shifts for ^{1}H NMR spectra are reported in parts per million relative to the signal of residual $CHCl_3$ at 7.27 ppm. Chemicals shifts for ^{13}C NMR spectra are reported in parts per million

relative to the center line of the $CDCl_3$ triplet at 77.23 ppm. The abbreviations s, d and m stand for the resonance multiplicity singlet, doublet and multiplet, respectively. IR spectra were obtained from Nicolet 380 FT-IR spectrometer. Mass spectra were recorded at the Mass Spectrometry facility in the Department of Chemistry of the University of Utah.

Reaction procedure and characterization of the product



2.28

Synthesis of indole DKP bridge 2.28. To a solution of **2.14** (0.046 g, 0.142 mmol) in THF (4 ml) at -78 ^oC, LHMDS (0.17 ml, 0.17 mmol) was added and stirred for 1.5 min. A solution of **2.27** (0.1 g, 0.274 mmol) in THF (2 ml) was added for 2 min. The mixture was stirred at -78 ^oC for 0.5 h and slowly warmed to RT. After stirring at RT for 1 h, the reaction was quenched with saturated NaCl and extracted the aqueous phase with CH_2Cl_2 (3 x 25 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The crude material was purified by silica flash column chromatography (4:6 EtOAC: Hexane) to afford 0.021 g (25%) of **2.28** as a yellowish oil. (R_f = 0.45 in 4:6 EtOAC: Hexane).

^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.4 Hz, 1 H), 7.62 (d, *J* = 8.4 Hz, 2 H), 7.44 (d, *J* = 8.4 Hz, 1 H), 7.33 (d, *J* = 8.8 Hz, 2 H), 7.29 (d, *J* = 8.4 Hz, 1 H), 7.25- 7.21

(m, 2 H), 7.17 (d, *J* = 6.8 Hz, 2 H), 6.85 (d, *J* = 8.8 Hz, 2 H), 5.22 (s, 1 H), 5.09 (s, 1 H), 3.91 (d, *J* = 16.5 Hz, 1 H), 3.79 (s, 3 H), 3.21 (d, *J* = 16.5 Hz, 1 H), 3.16 (s, 3 H), 2.98 (s, 3 H), 2.31 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 164.6, 160.9, 145.2, 135.1, 134.9, 130.9, 130.7, 130.1, 126.9, 126.7, 125.4, 124, 123.7, 118.9, 116.4, 114.6, 114.2, 71.2, 66.3, 55.6, 50.9, 31.1, 30.6, 28.1, 21.8. IR (neat): 2922 cm^{-1}, 2852 cm^{-1}, 1685 cm^{-1}, 1173 cm^{-1}. LRMS (ESI) *m/z* calc'd for $C_{30}H_{29}N_3O_5S_3$ (MNa+): 630.1, (MK+): 646.1; found (MNa+): 630.1, (MK+): 646.1.

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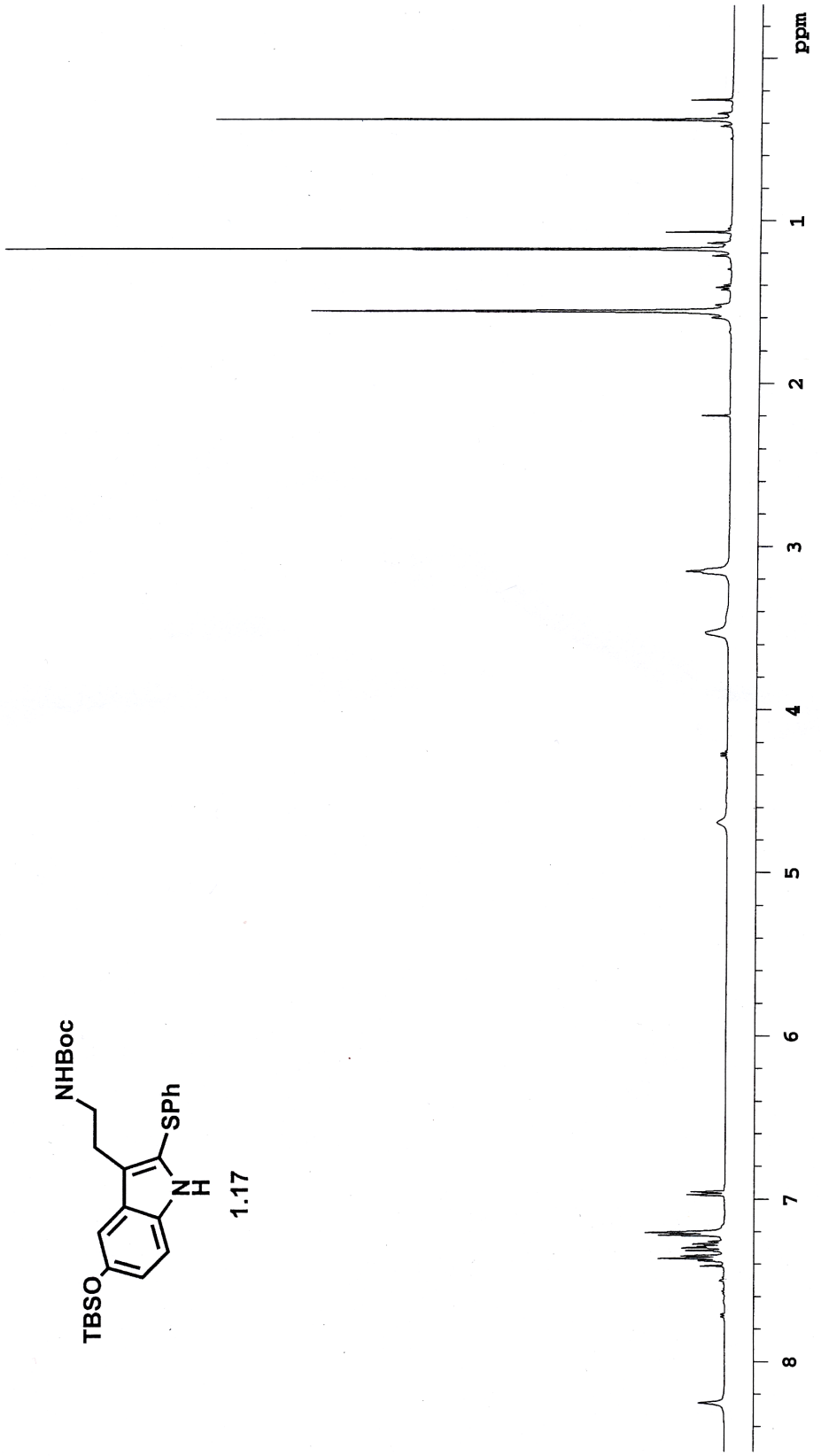
APPENDIX

^{1}H AND ^{13}C NMR SPECTRA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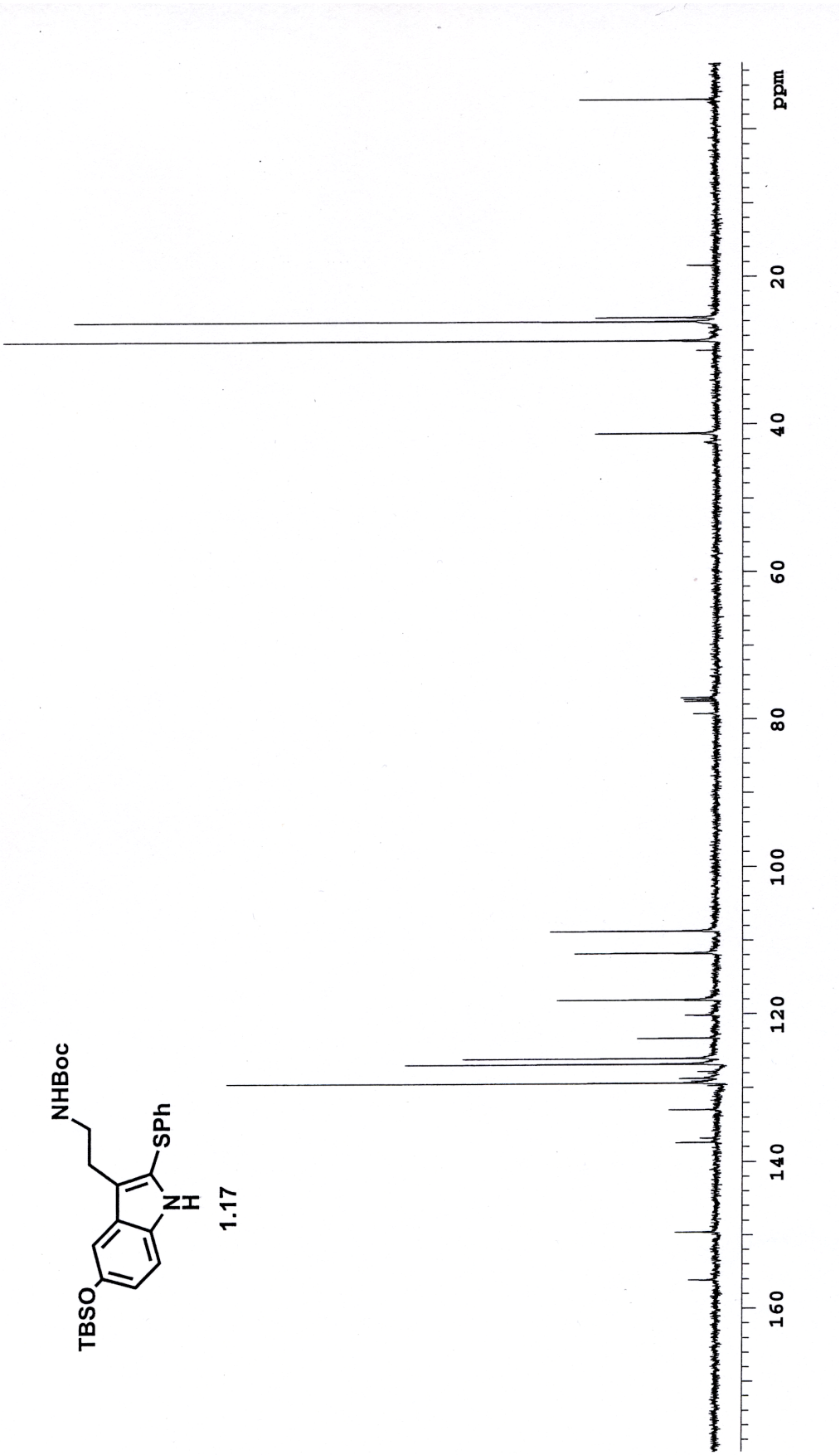
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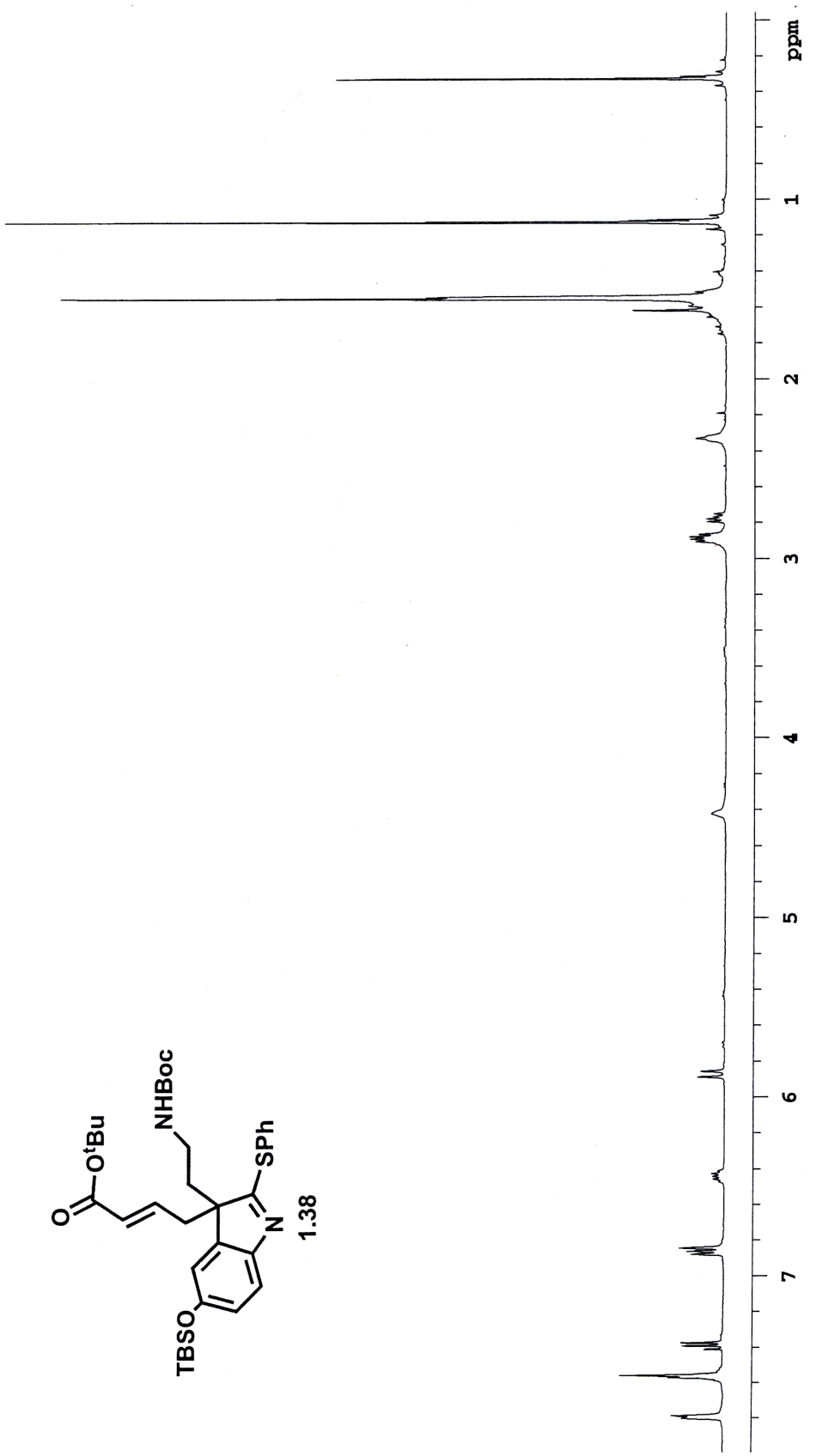
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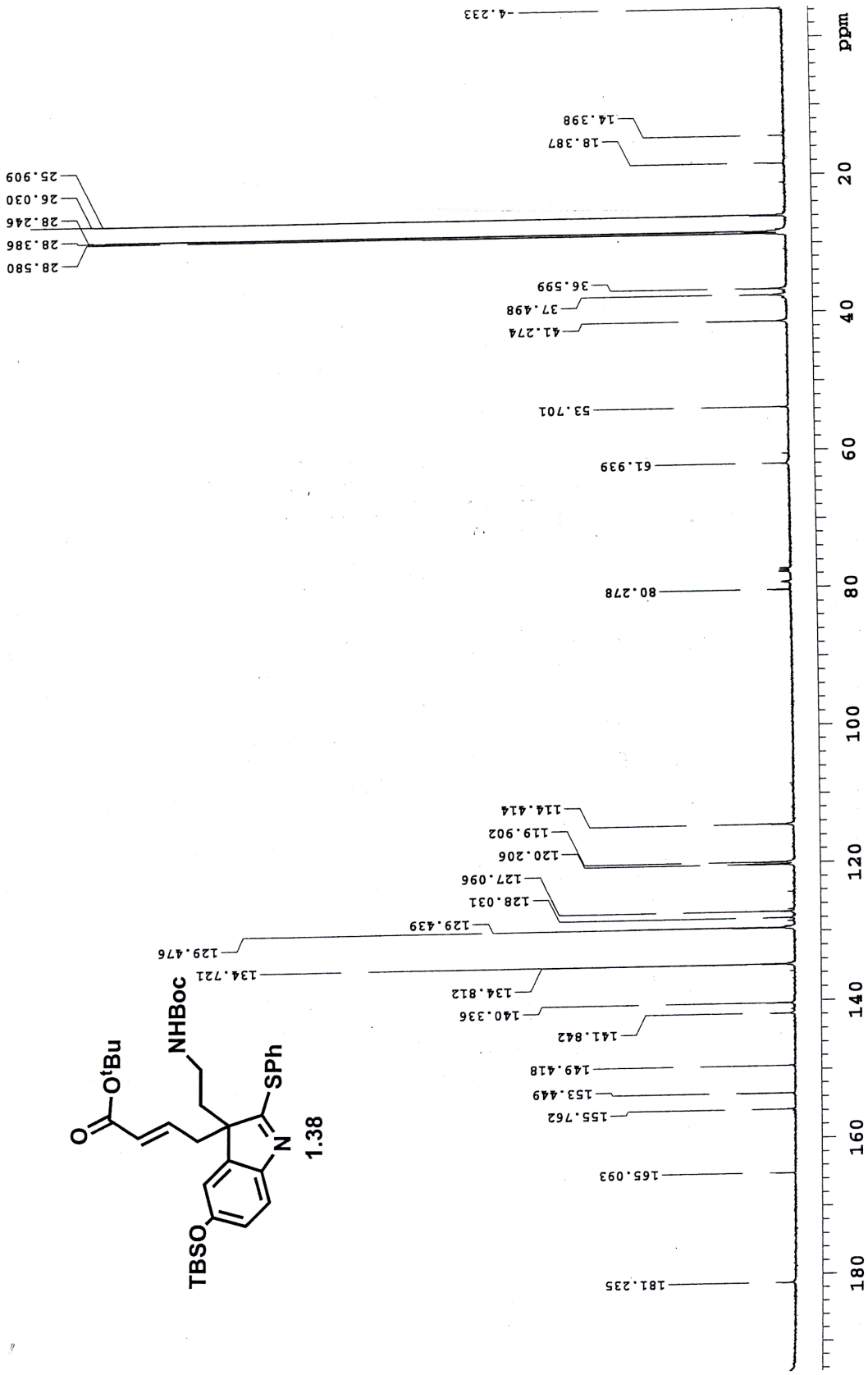
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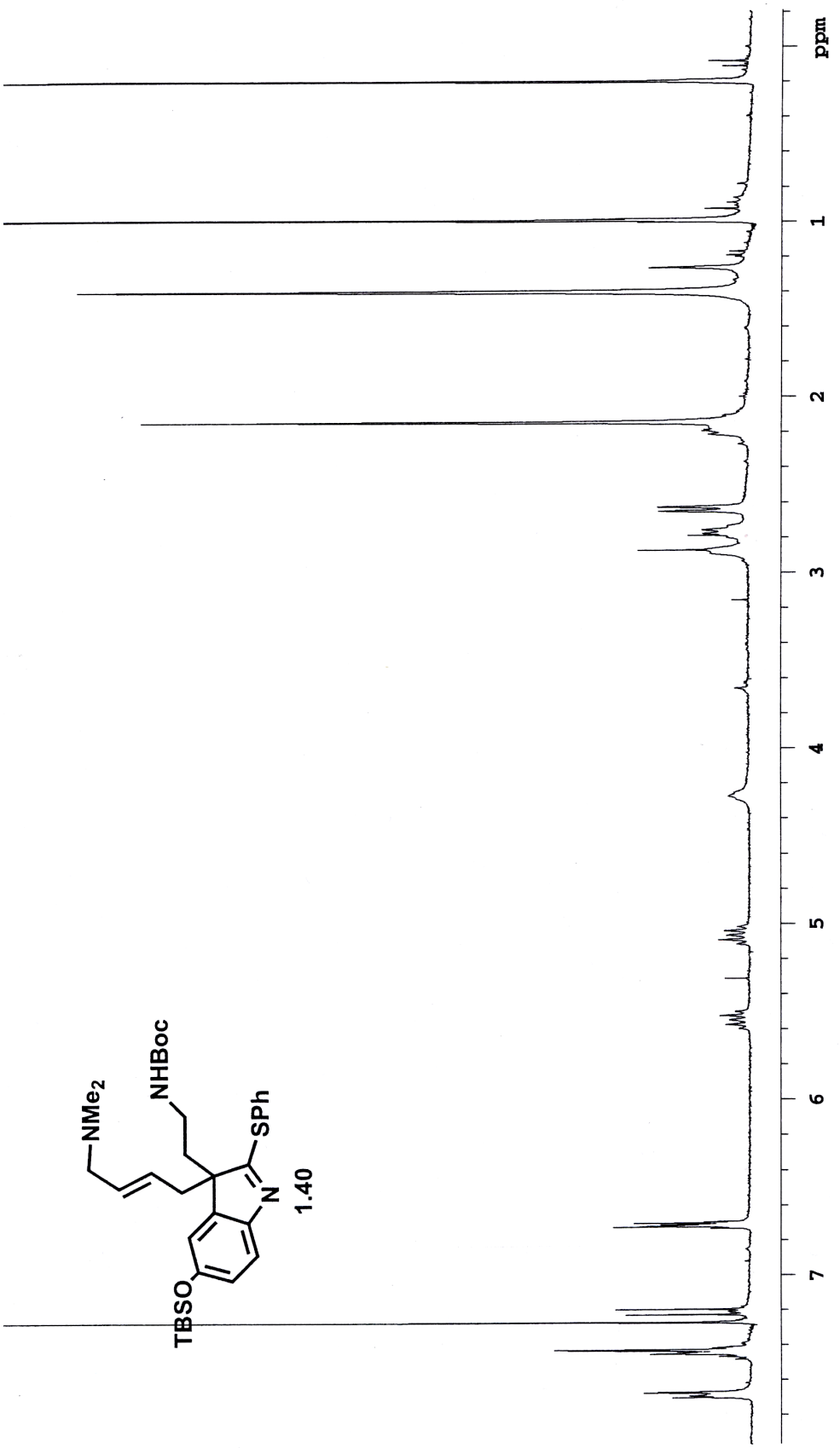
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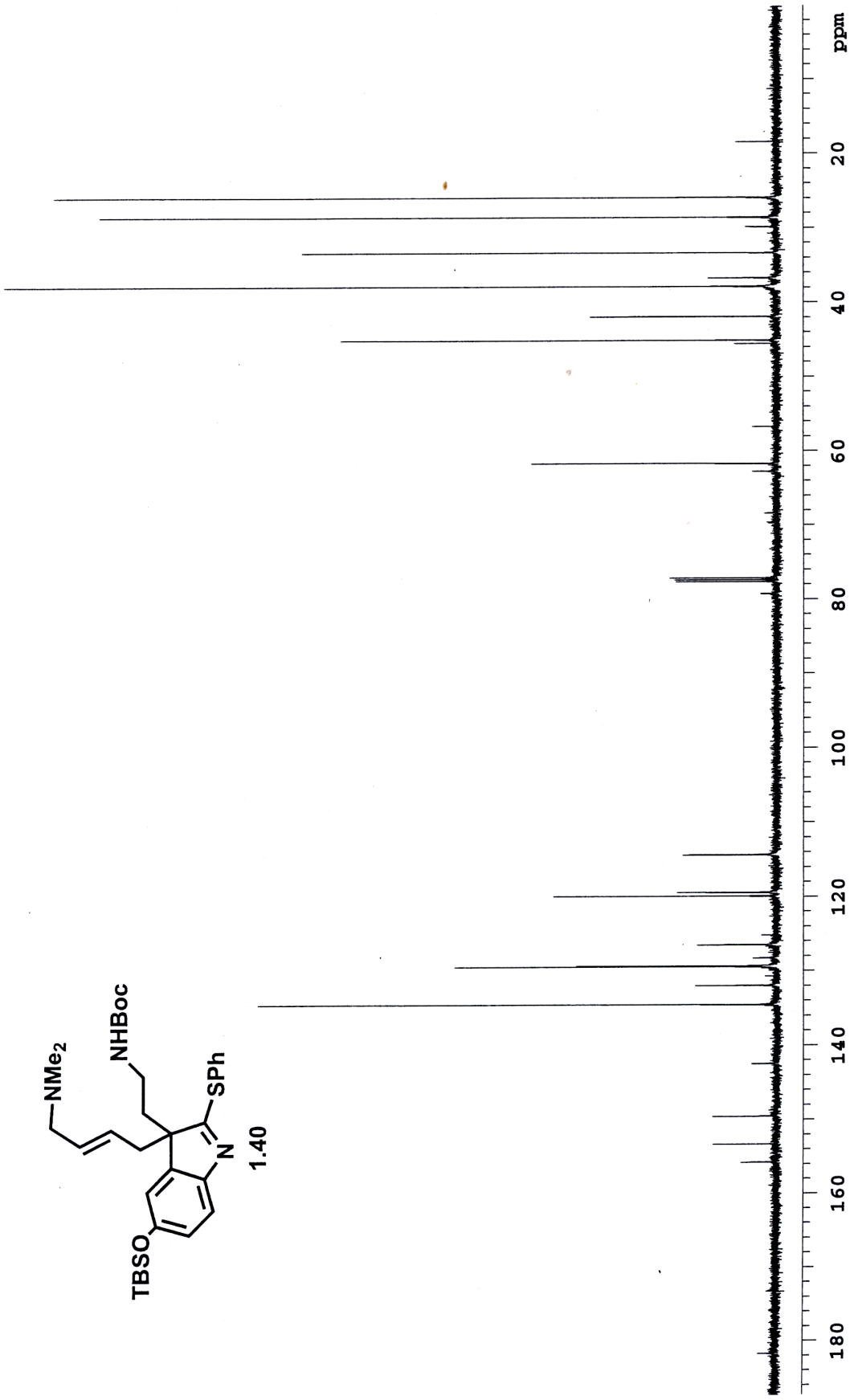
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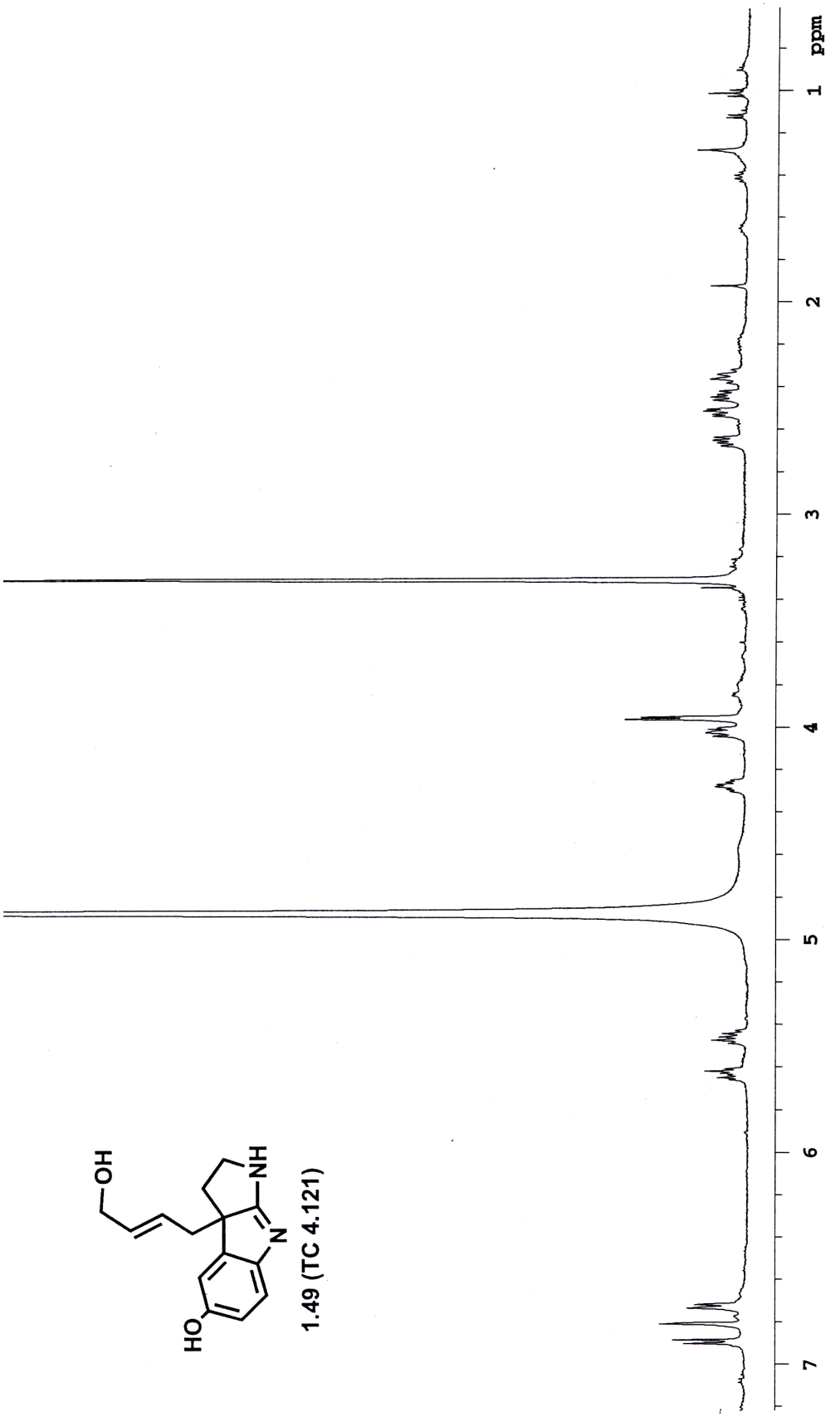
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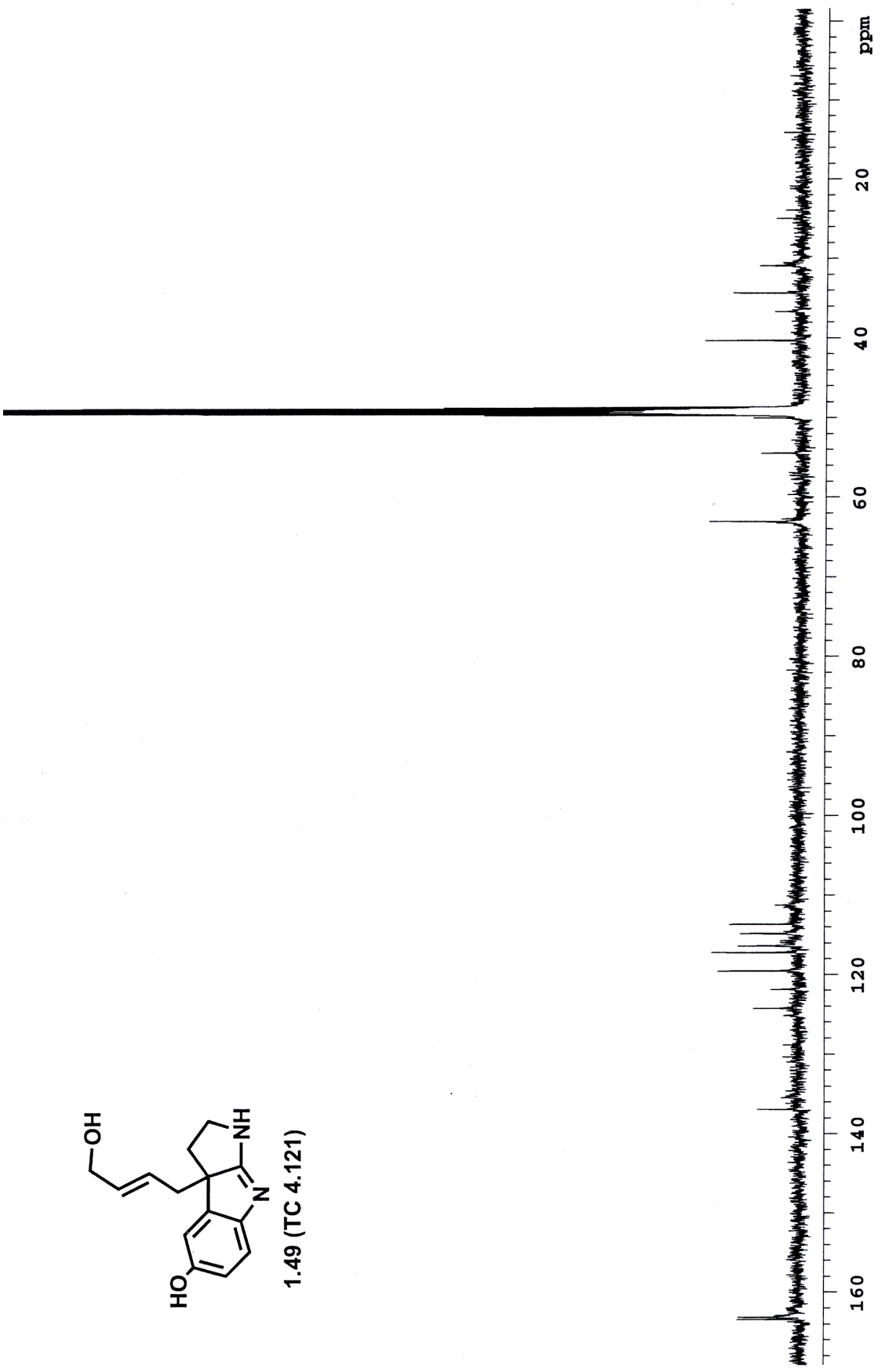
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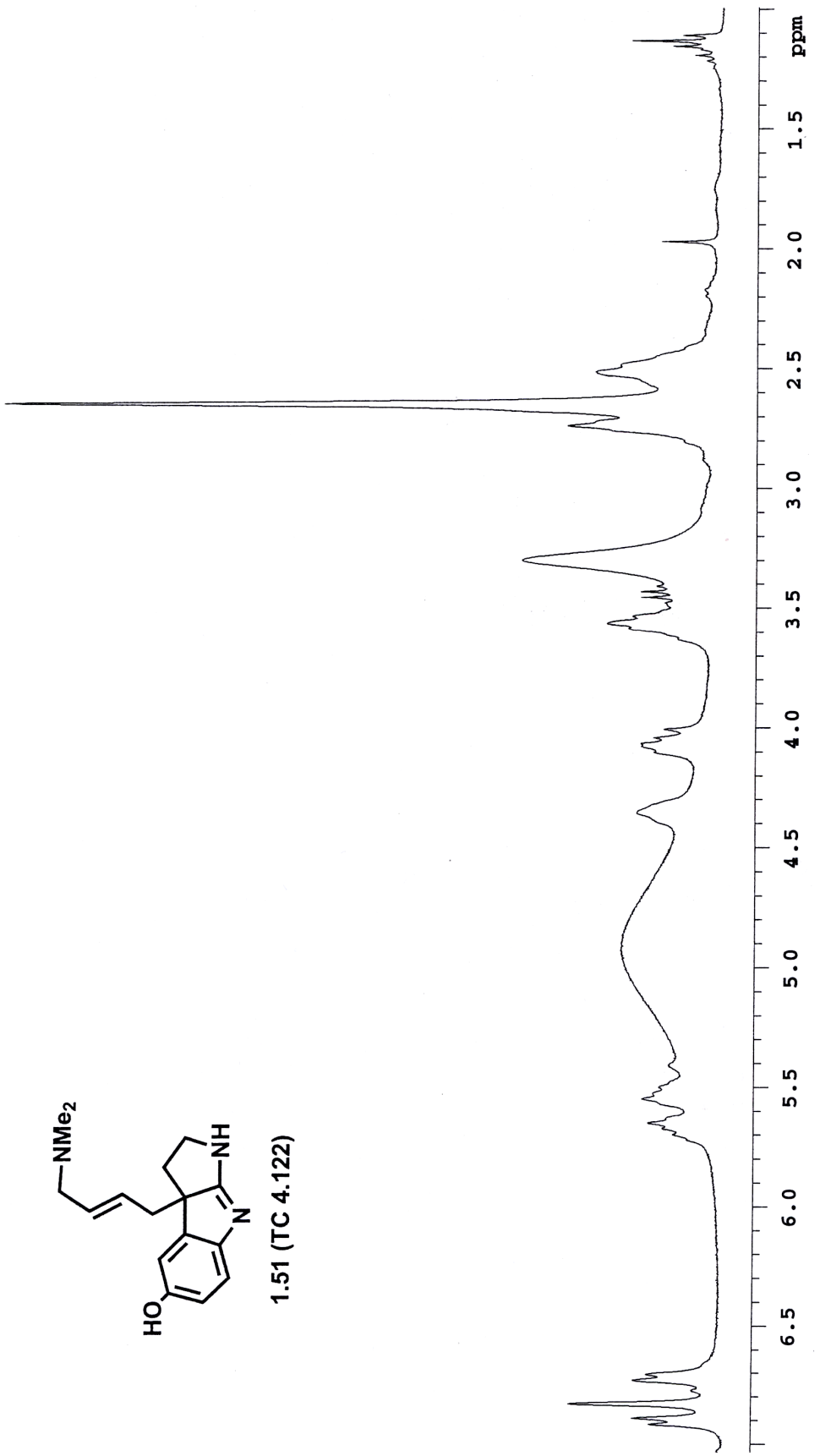
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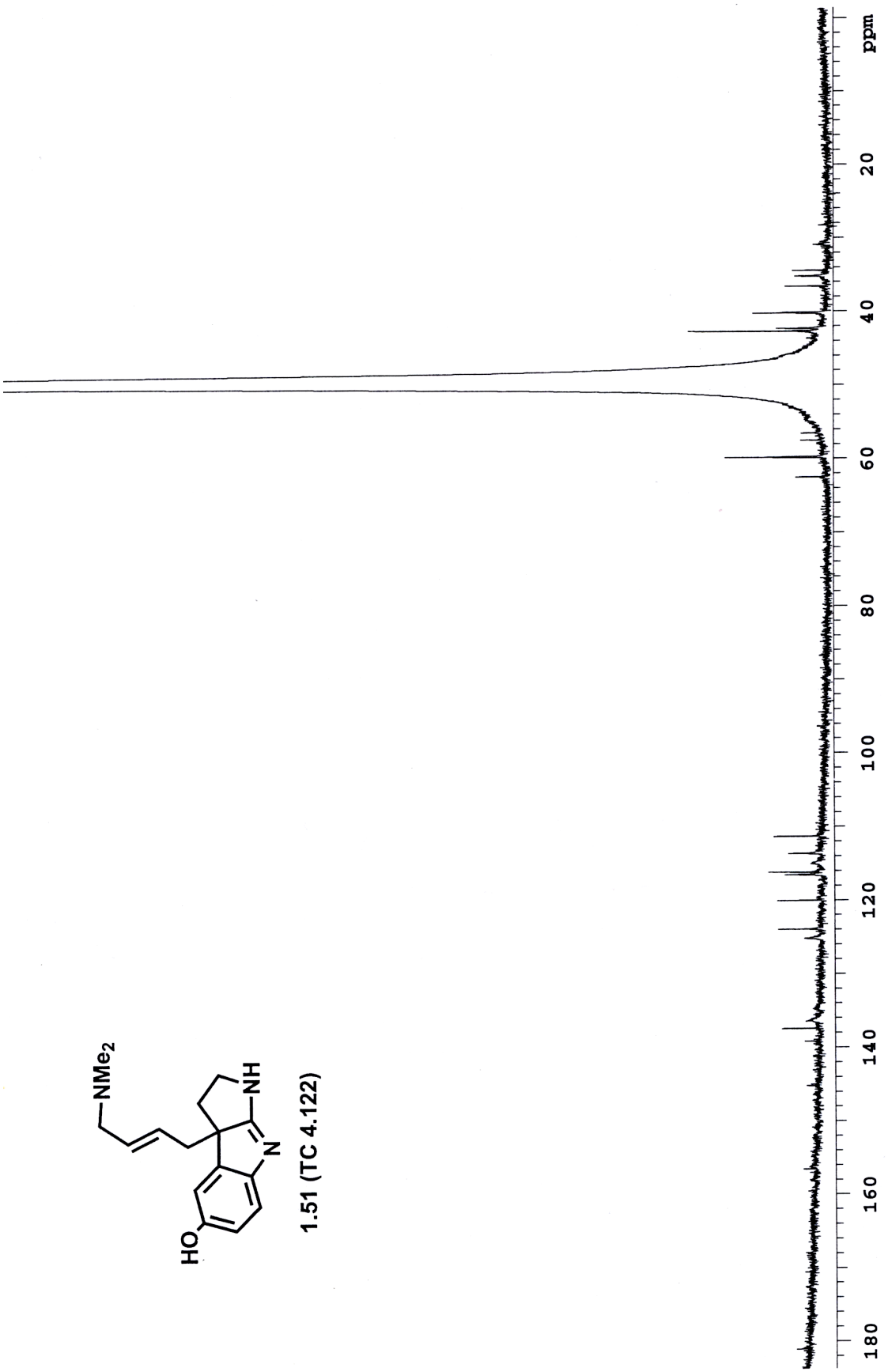
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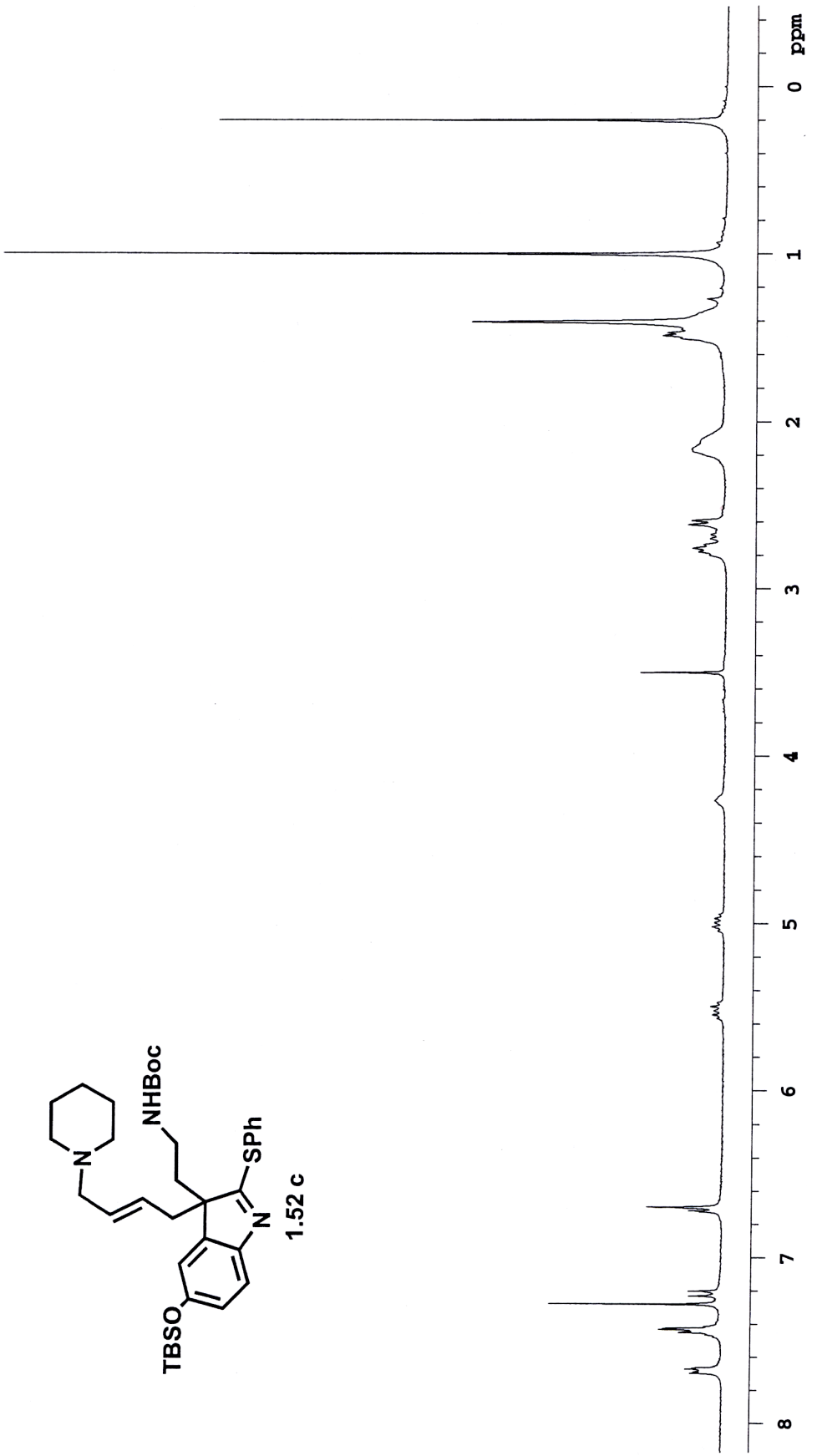
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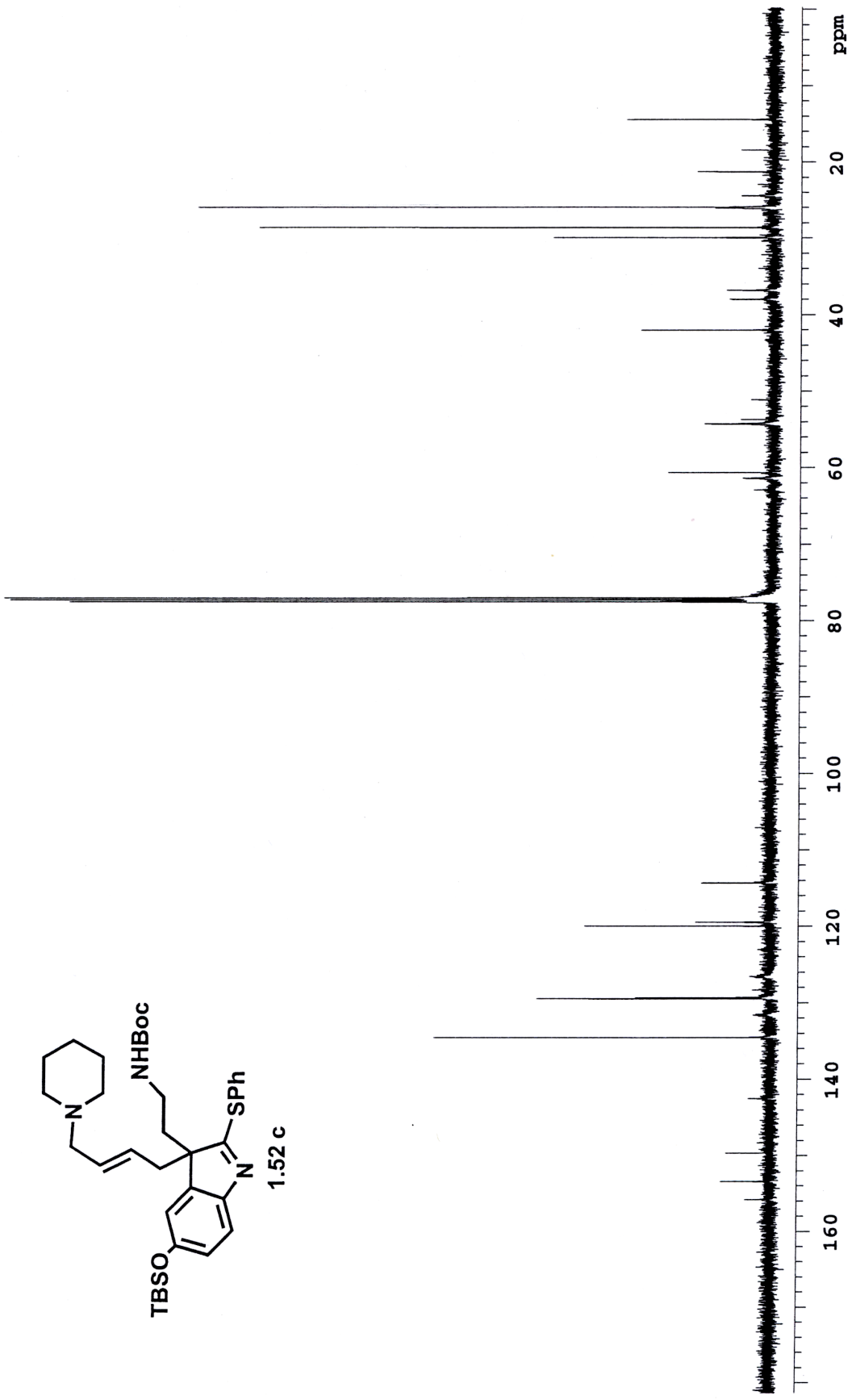
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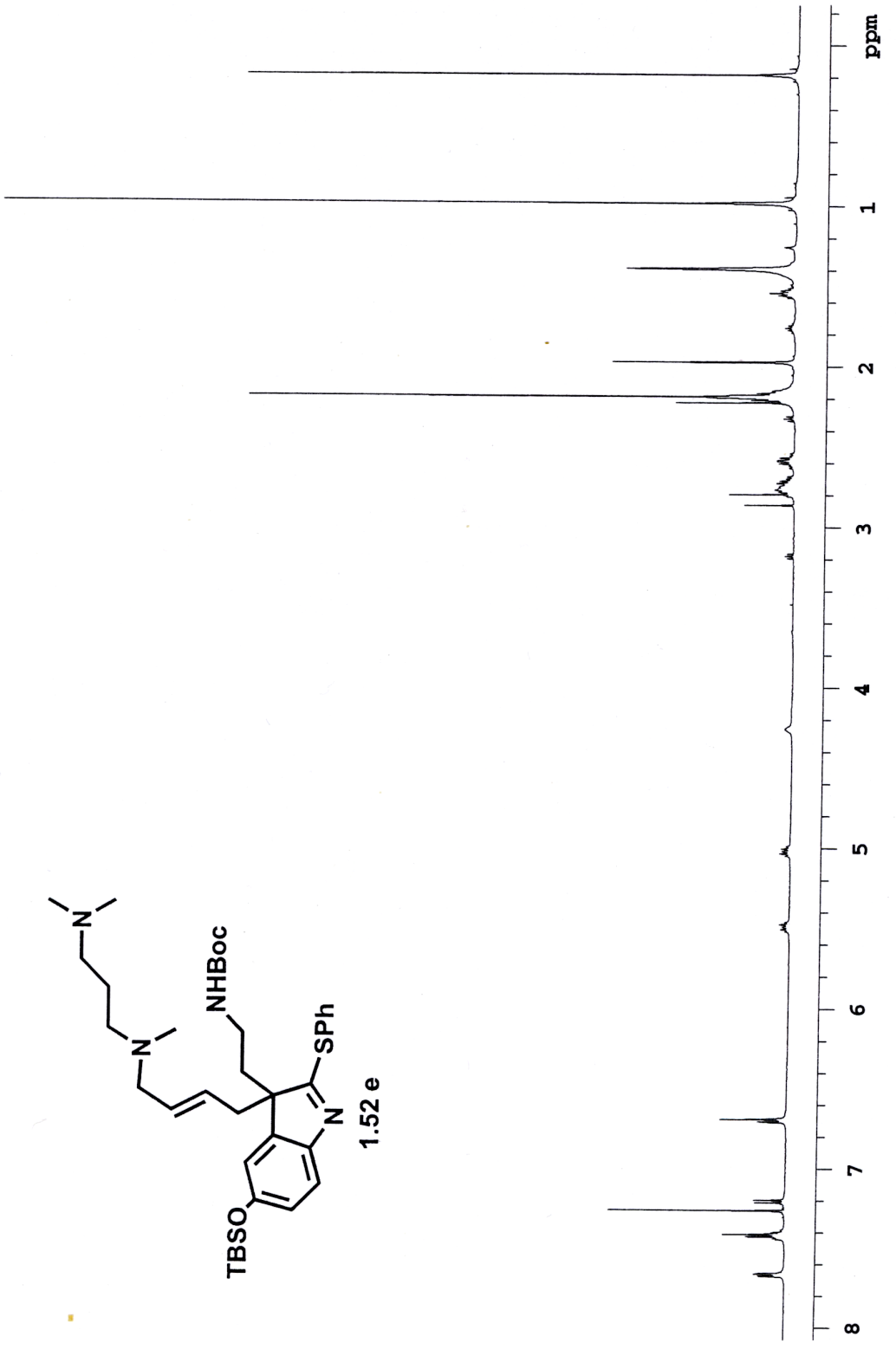
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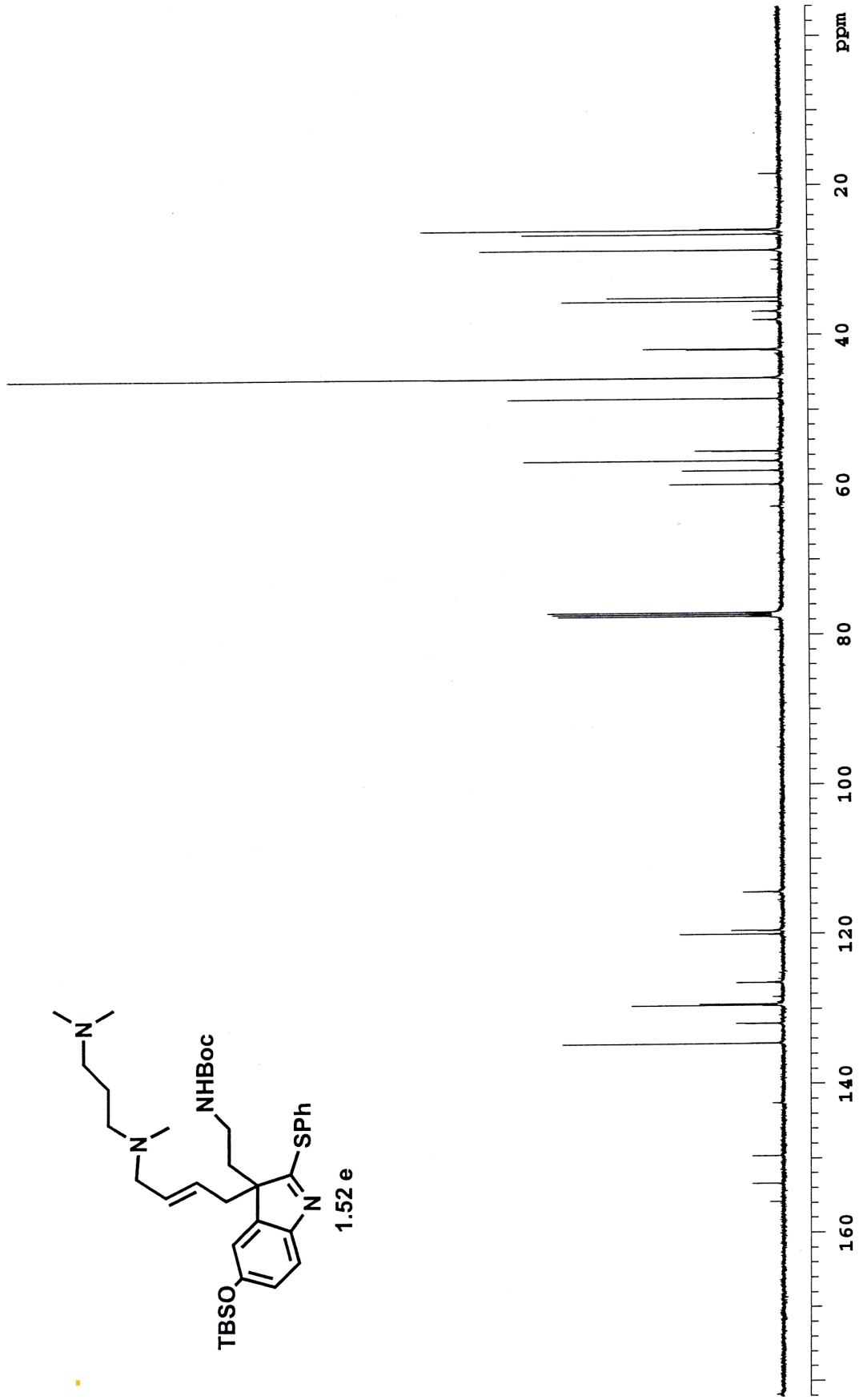
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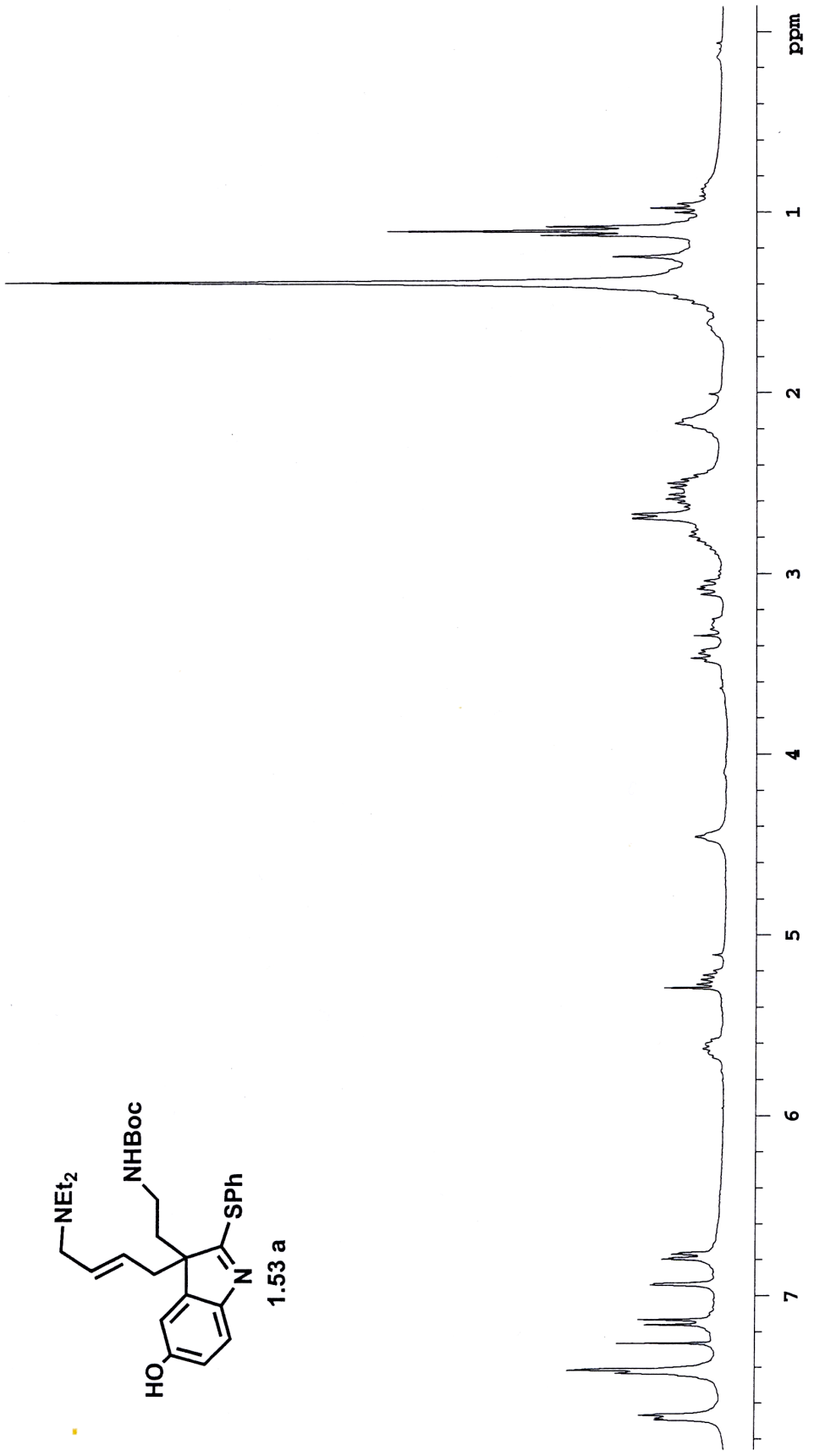
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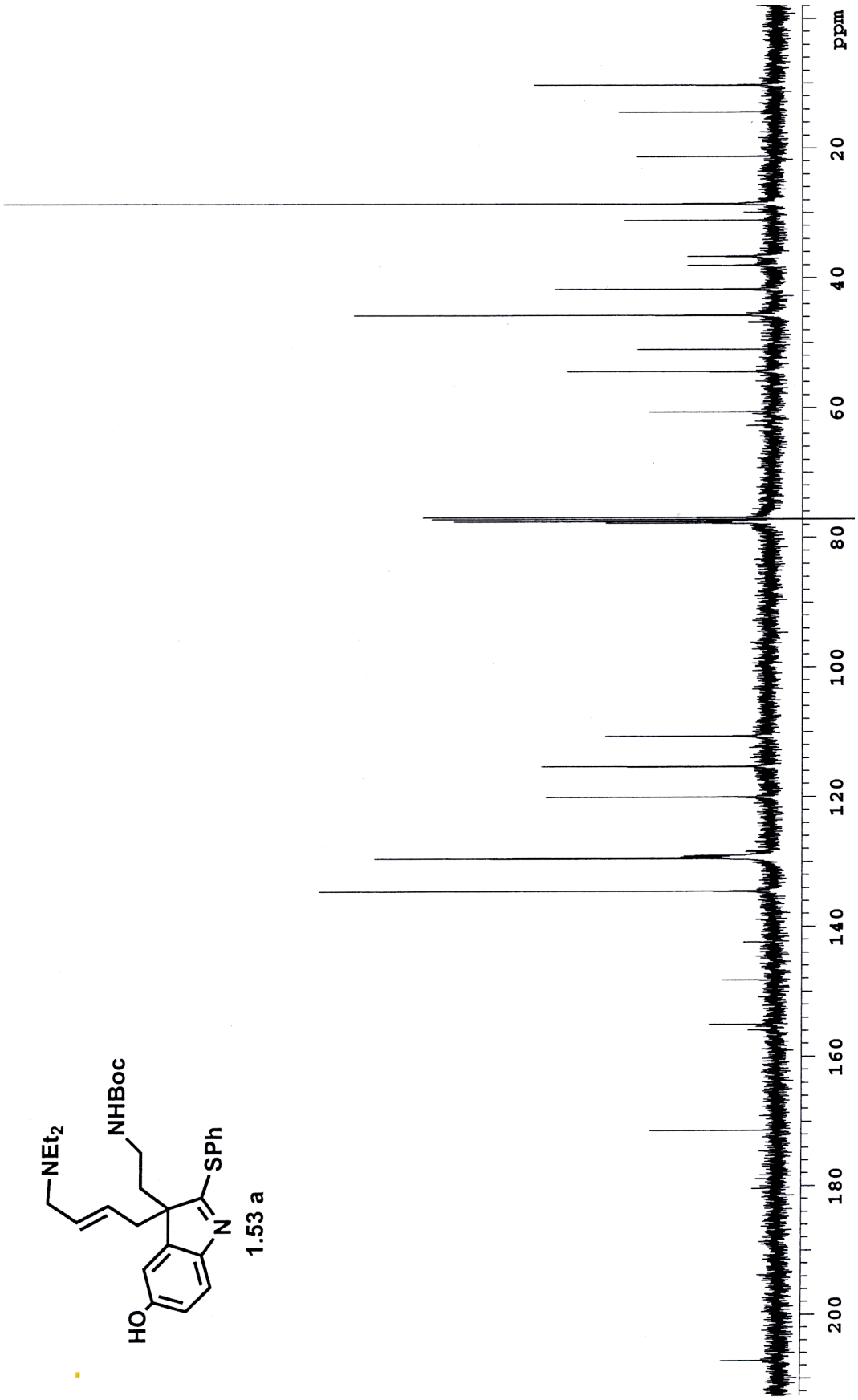
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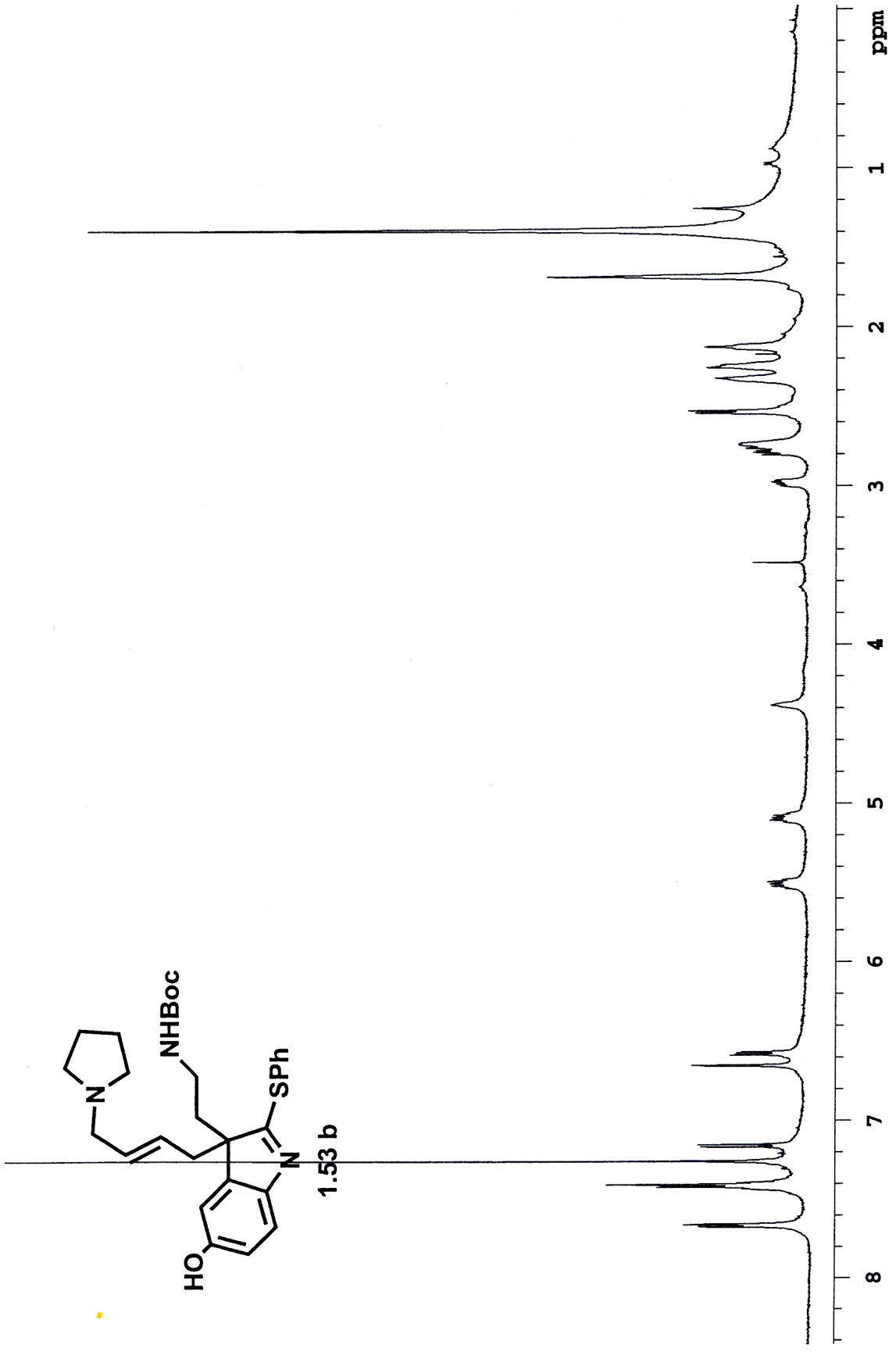
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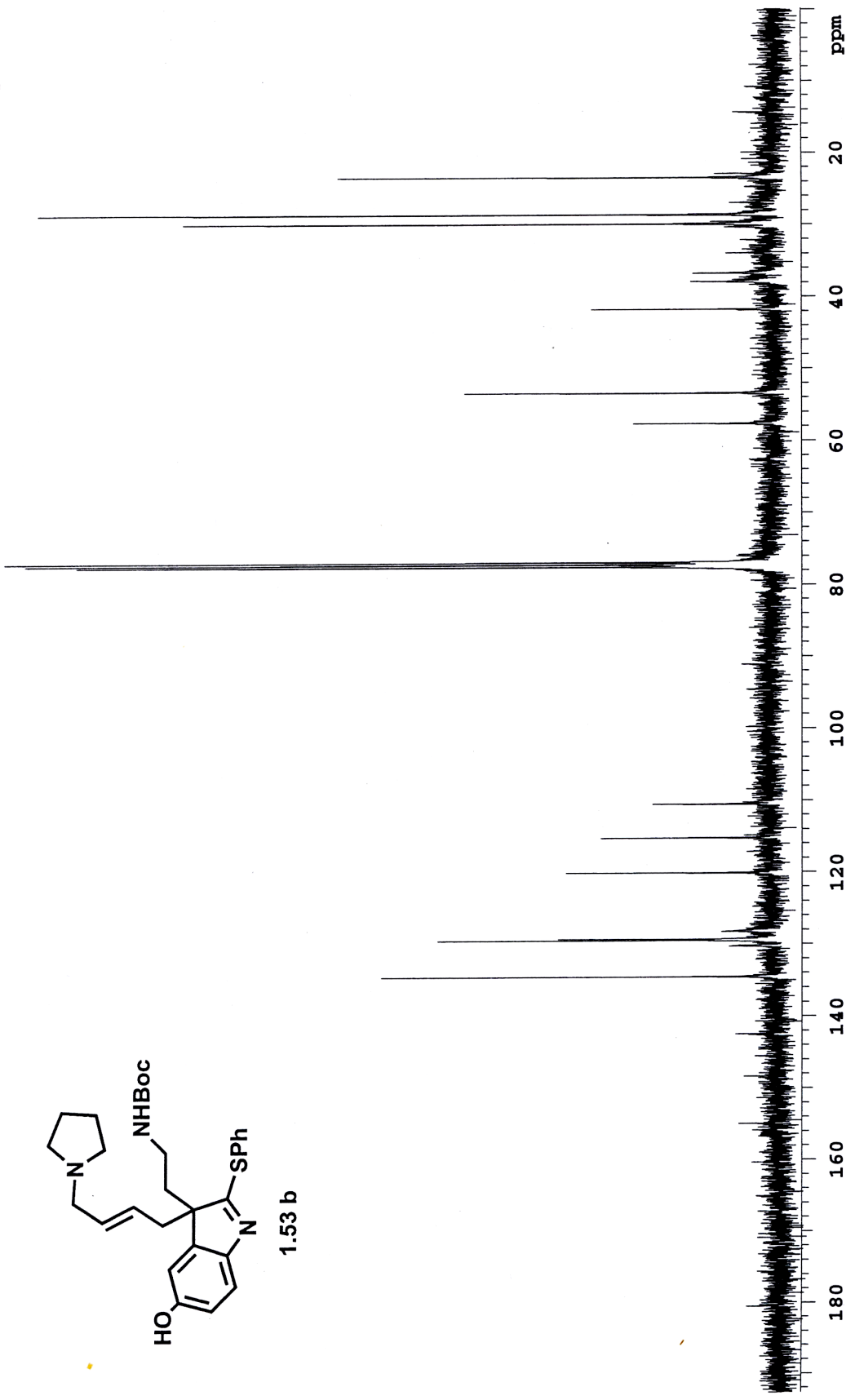
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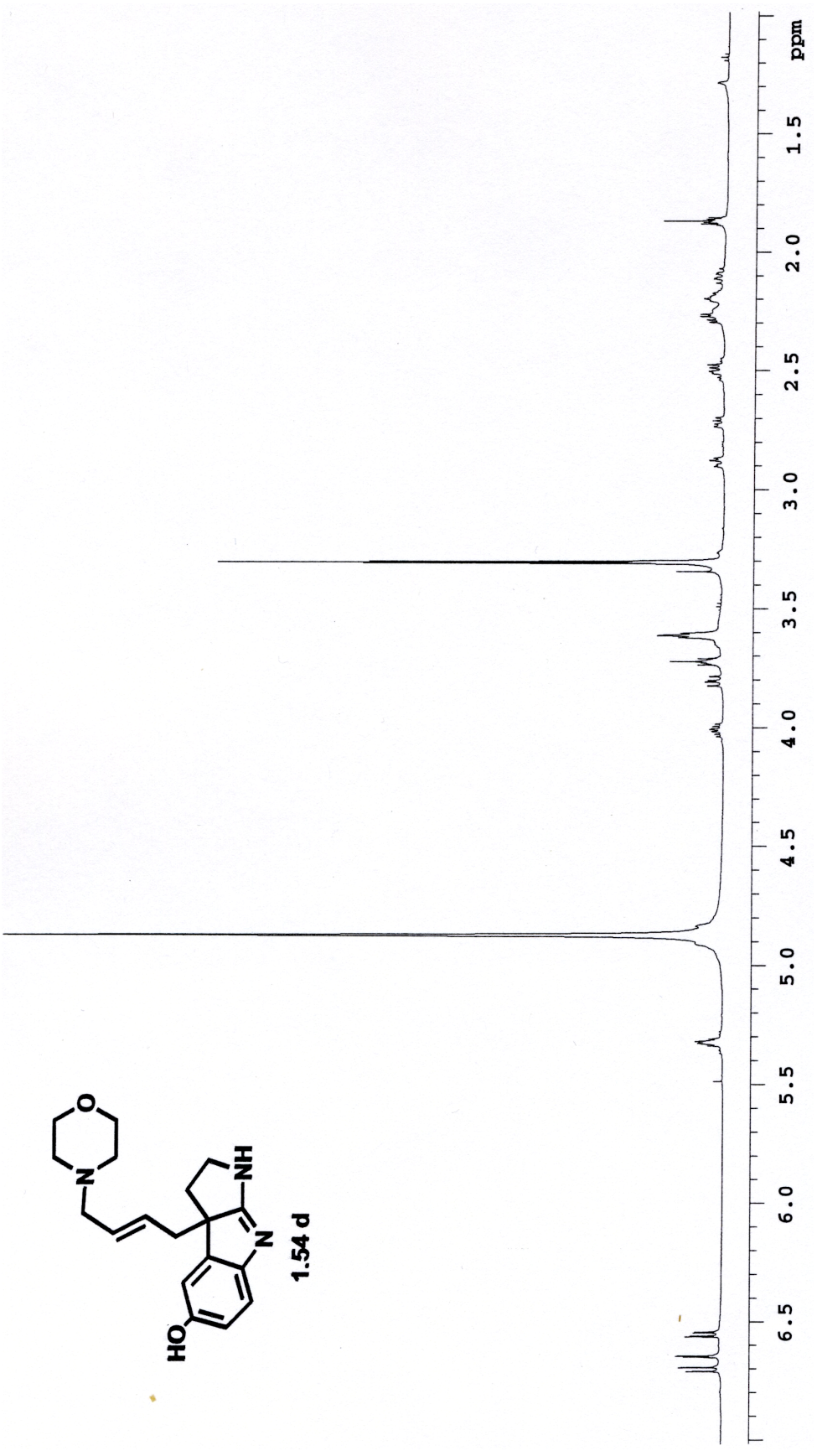
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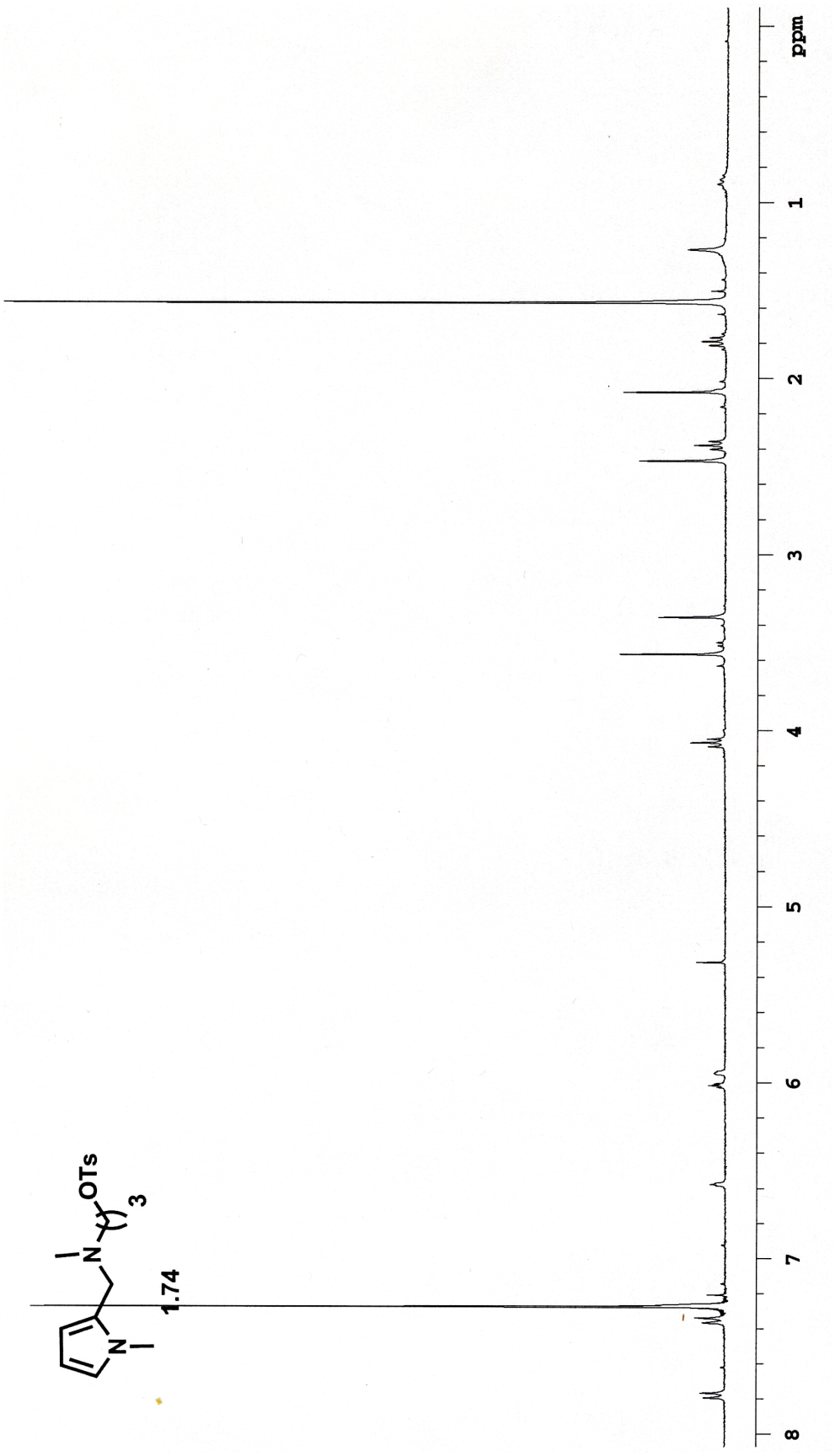
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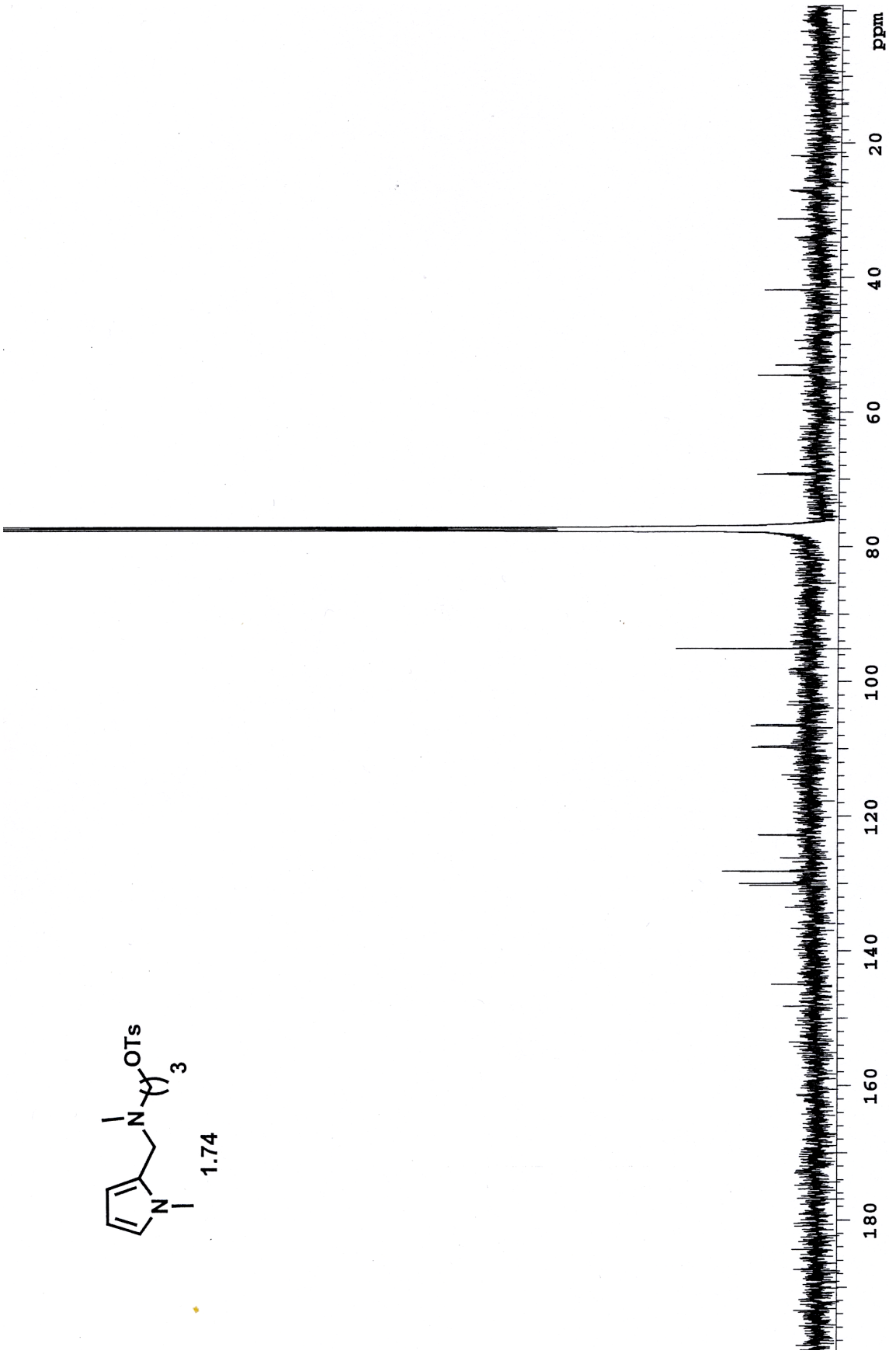
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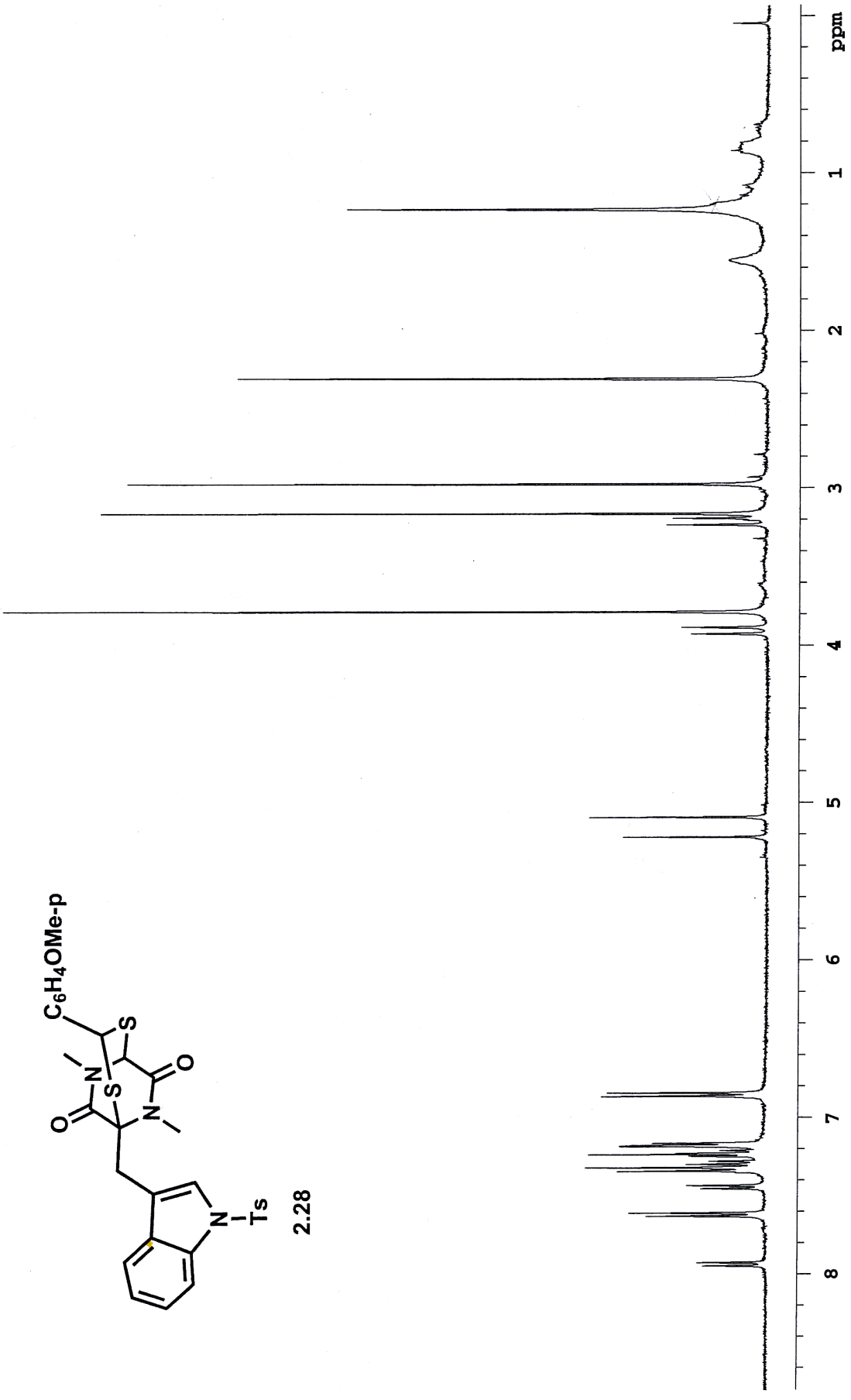
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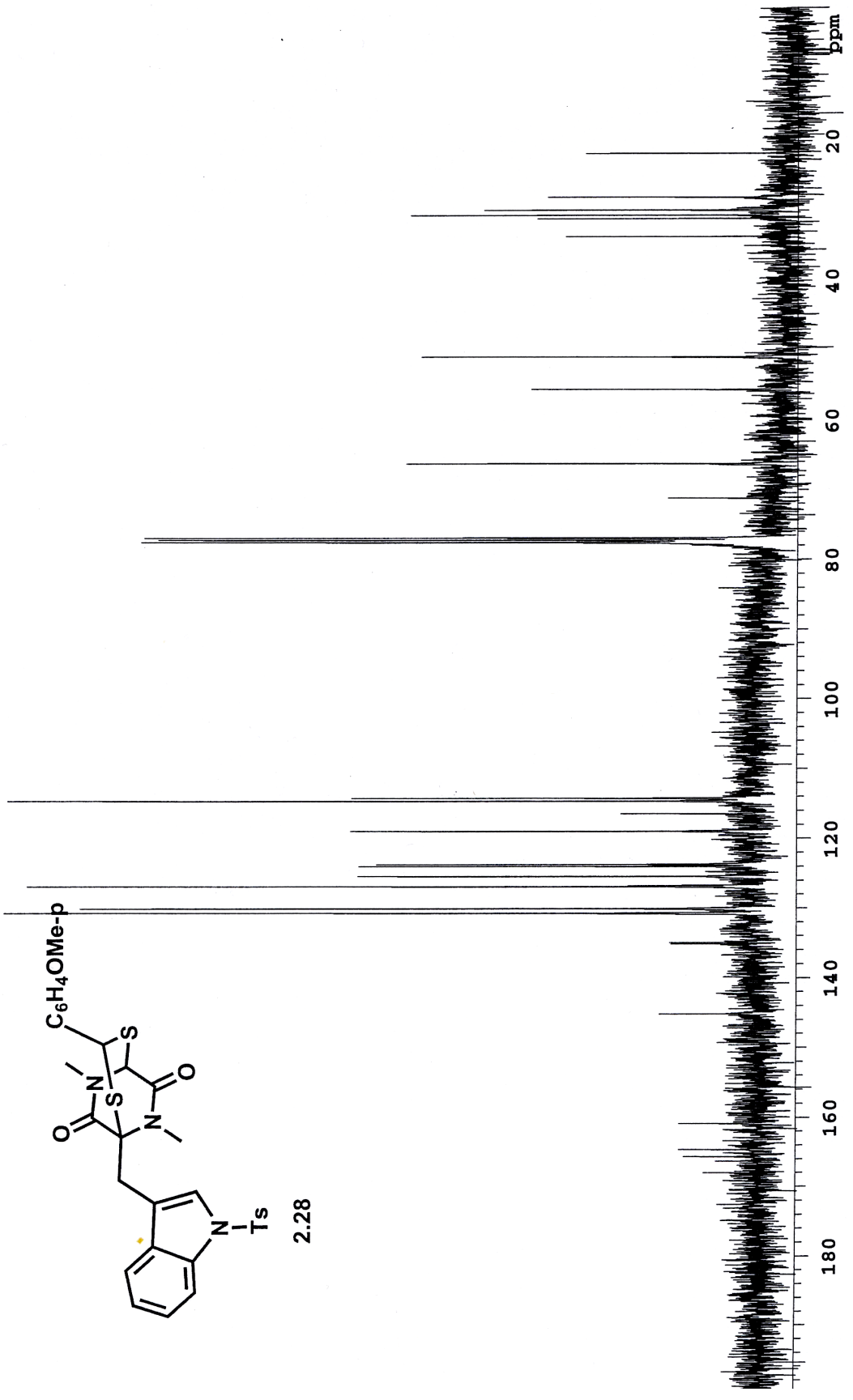
N
S
S
N
O
N
Ts
2.28
ppm
1
2
3
4
5
6
7
8


C6H4OMe-p
N
S
S
O
O
N
N
Ts
2.28
ppm
180
160
140
120
100
80
60
40
20